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Devoted to the Advancement of the Biological Sciences
With Special Reference to the Factors of Evolution

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THE AMERICAN NATURALIST

A MONTHLY JOURNAL

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AMERICAN NATURALIST

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SOME FUNDAMENTAL MORPHOLOGICAL OBJECTIONS TO THE MUTATION THEORY OF DE VRIES

PROFESSOR EDWARD C. JEFFREY

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THE hypothesis of the saltatory origin of species has received a new impetus from the investigations of De Vries,* published in his "Mutationstheorie" and subse-

[1] To an address delivered in Brussels before the outbreak of the war and published in Science (Vol. 40, No. 1020, July 17th.), Professor de Vries appends a criticism of the writers preliminary article on mutation, likewise published in Science (Vol 39, No. 1005, April 3d.). The gist of his objection to the writer's position, that Œnothera and other members of the Onagraceæ are in a position of hybrid contamination, as evidenced by the frequent sterility or partial sterility of their pollen, is the contention that pollen sterility and gametic sterility in general is not sufficient evidence of hybrid contamination. To this statement two replies may be made. In the first place prominent geneticists for many years have recognized pollen sterility as important evidence of hybridization. Secondly investigations, which have now become very extensive, on the Angiosperms as a whole, show very interesting conditions in many natural families. While the monotypic species and those which are isolated geographically or phenologically (that is by a time of flowering later or earlier than that of the mass of species belonging to the genus) have invariably good pollen, those species, which overlap in their geographical range and in their times of flowering in many cases are characterized by abortion of the reproductive cells. In other words pollen infertility is only found where the possibility of crossing is present. This principle has been illustrated in the body of the present article by reference to the Rosaceæ. Taking a further illustration from the large family Ranunculaceæ, *Ranunculus acris* and *R. repens,* which overlap both in range and time of flowering have pollen, which is often largely imperfect, particularly in the first mentioned species. *R. rhomboideus* on the other hand, flowering in the very early spring has perfect pollen development.

quent works. The chief foundation for his views, in regard to the instantaneous origin of species, is furnished by the conduct of *Œnothera lamarckiana* in cultures. It has been somewhat generally recognized that *O. lamarckiana*, and more recently, other species of the genus as well, constitute crucial evidence in regard to the validity of the mutation hypothesis on the botanical side. A great many investigations on the genetics and cytology of *O. lamarckiana* and other species, as well as crosses between species and "mutants" of *Œnothera*, have been carried on during the past decade by De Vries, and his followers and opponents. As a result a huge and highly technical literature has grown up. *Œnothera* is obviously regarded, on the botanical side at any rate, as the touchstone of the mutation hypothesis as formulated by De Vries. Obviously if this genus does not stand the test of critical investigation, the mutation hypothesis, so far as its validity depends upon De Vries's chosen illustration, is discredited.

Since *Œnothera* and by obvious implication the Onagraceæ, to which it belongs, have become authority for the mutation hypothesis, in its latest revival, they must like Cæsar's wife be beyond suspicion. Like Cæsar, *Œnothera* has become a name of authority and its family affairs accordingly, should be beyond suspicion, when subjected to the most searching investigation. It is apparently just in this direction that the weak spot of the mutation hypothesis lies. Too much attention has apparently been given to ringing the changes on the so-called mutants of *Œnothera* and not enough to the investigation of the general morphological situation in the Onagraceæ, to which this much-discussed genus belongs.

Unusual variability in plants is ordinarily regarded as *prima facie* evidence of hybridism and the suggestion has in fact frequently been made by professional geneticists (*e. g.*, Bateson, Davis, East, Gates and others) that *Œnothera lamarckiana* is a hybrid. It is perhaps of interest in this connection to recall that one of the commonest expedients adopted by the practical breeder, for breaking

up the continuity of the germ plasm, is hybridization. Apposite in this connection is the wholesale hybridizing practised by Burbank, for the purpose of bringing about the necessary genetic plasticity in his cultures and thus obtaining by resultant mutation or variation, new and desirable varieties of useful plants. The morphological peculiarities of hybrids have been clearly recognized for nearly a hundred years. They are for example clearly set down in Gaertner's rare and classic prize essay, entitled "Versuche und Beobacthungen ueber die Bastarderzeugung im Pflanzenreich" (Stuttgart, 1849). Curiously enough these important criteria have been largely ignored by the adherents of the mutation hypothesis of De Vries. A very important and generally observed difference between hybrids and genetically pure species, is the very easily detected one of pollen sterility, partial or complete. Of course when the hybridizing forms show a considerable degree of compatibility, this character may be inconspicuous or even absent. Further even in cases where it is originally present, it may be subsequently largely eliminated by selection. De Vries himself has noted that about one third of the pollen of *O. lamarckiana* is abortive. The English geneticist Bateson was struck with this peculiarity of the species, so much discussed in recent years, in relation to its variable offspring in cultures and promptly and first called attention to the obvious significance of this feature, suggesting that *O. lamarckiana* was a hybrid and that its remarkable conduct was the result of hybridization. This objection has in reality never been met. It is the purpose of the present article to show on grounds commonly accepted by geneticists and morphologists, that not only is genus *Œnothera* in general characterized by genetically impure or hybrid species, but that the condition of genetical impurity is extremely common in the Onagraceæ as a whole.

It will be convenient to begin with the examination of our common and very variable garden *Fuchsias*, which belong to the family Onagraceæ. The common *Fuchsia*,

sometimes known to gardeners as *Fuchsia speciosa,* is recognized as a hybrid derivative of *Fuchsia magellanica,* a native of southern South America. Fig. 1 illustrates

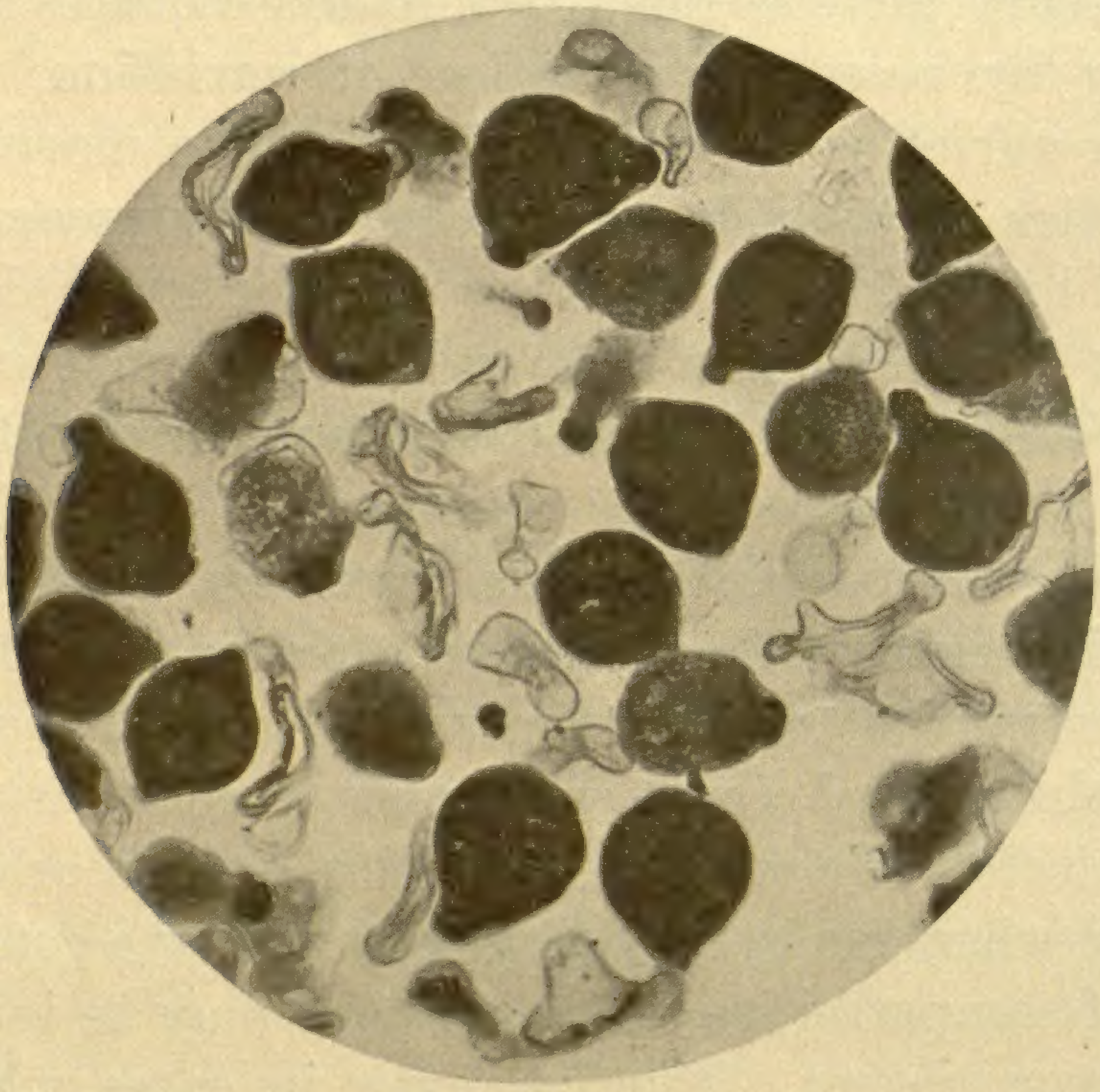


FIG. 1

photomicrographically, the condition of the pollen in one of the garden varieties of *Fuchsia.* The sound pollen grains appear as dark bodies with two or more germination pores projecting from their surfaces. The dark color of the grains is due to the deeply staining character of their protoplasmic contents. More than a third of the pollen present in the anther cavity is abortive and is represented in the photograph by shrivelled light-colored objects, which are in fact empty and collapsed pollen grains. In other varieties of the garden *Fuchsia,* the grains may either be entirely abortive and empty (as is the case for example in the so-called mutant of *Œnothera lamarckiana,* known as *O. lata*) or they may all be more or less well developed so far as their protoplasmic contents are concerned, but extremely varying in size. In the present description, perfection or imperfection of pollen is judged only from the morphological aspect, because this is the significant point of view from the standpoint of the

detection of hybridization. Physiological sterility is frequently due to entirely different causes than genetical lack of harmony, as for example in the horseradish or the potato (*Solanum*). In the former it has been found possible to bring about the formation of fertile seed by simply girdling the top of the subterranean storage region of the plant, so as to prevent the undue descent of assimilates. The common white lily, *Lilium candidum*, presents a similar condition, for here the setting of seed takes place only when the leafy flowering axis is severed from its bulb and kept in water. So far as I am aware, there have been no experiments as to the result of severing the continuity of the phloem (girdling), in relation to the restoration of seed production in the potato. The common yellow day lily (*Hemerocallis*) possibly presents a case similar to that of *Lilium candidum*, for it does not ordinarily set seed, although in all the examples I have examined the pollen was morphologically perfect. I have not yet been able to secure flowers of any pure species of *Fuchsia*, a genus which flourishes mostly in the remoter parts of South America and in the New Zealand islands. The cultivation of *Fuchsias*, although once very popular, has now gone out of vogue and it is consequently difficult to secure specimens of the species. As has been pointed out the commonly cultivated *Fuchsias* are of hybrid origin.

We may now turn our attention to a very puzzling genus of the Onagraceæ, namely *Epilobium*. This genus has been a great riddle to systematists and the determination of species has been extremely difficult on account of their extreme variability. In European systematic works, this high degree of variability is recognized clearly to be largely due to hybridization and in such a standard work as the "Naturliche Pflanzenfamilien" of Engler and Prantl, the statement is definitely made that the various species of *Epilobium* frequently and commonly hybridize with one another in nature. Let us consider in this connection the northern hemisphere cosmopolitan species, known as *Epilobium angustifolium*, the willow herb or

fire weed, which by contrast to many of the other *Epilobiums,* is so constant and distinct that it is frequently referred to a separate genus, *Chamænerion.* This species shows its most marked distinction from other species of *Epilobium* (*Epilobium* proper) in the fact that its pollen grains are separate and not in tetrads, as is the case in other common species. Fig. 2 reproduces photograph-

FIG. 2

ically a transverse section of a mature flower bud of *E.* (*Chamænerion*) *angustifolium.* On the outside are seen the floral envelopes, two in number, composed, as is the rule in the Onagraceæ, of four parts each. Within lie four stamens represented by their anther sacks and internal to these are four stigmas representing the carpellary or ovarial portion of the flower. The photograph is on a sufficient scale of magnification to show the pollen grains in the loculaments or cavities of the anthers. Obviously the pollen is very uniform and perfect in its development. Fig. 3, likewise photographic, illustrates the organization of the pollen as viewed with a much higher magnification of the microscope. Although some of the grains are only

partially included in the plane of section, it is quite clear, that like those of *Fuchsia*, figured above, they have projecting germination pores, but unlike the *Fuchsia* of our illustration, all the pollen grains of *Epilobium* (*Chamænerion*) *angustifolium* are perfectly developed. I have examined the pollen of the species under discussion from widely separate geographical regions and under different conditions of growth and season, with the uniform result,

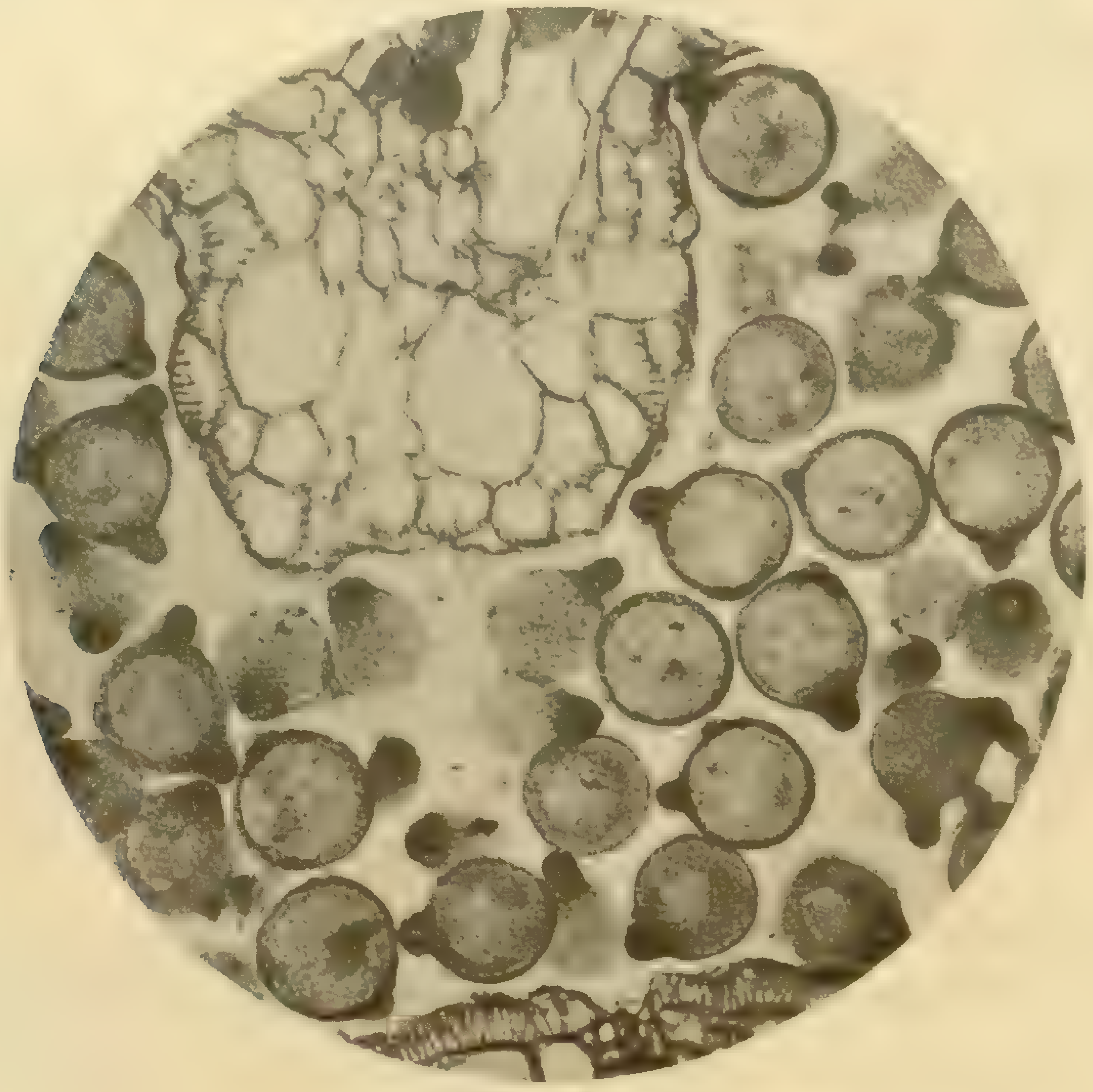


Fig. 3

that the pollen is perfect and invariable in any important respect. *E. angustifolium* is a species which apparently is not known to hybridize with other species and indeed it is not easy to see how it could cross with those having their pollen grains in tetrads. The perfection of the pollen in view of this condition appears particularly significant. The failure of *E. angustifolium* to hybridize in nature with other species of the genus is doubtless due to the fact that it is morphologically very distinct from these and would in all probability produce, if artificially crossed, only sterile hybrids.

We may now turn by way of comparison to a species of *Epilobium* of the ordinary type. Fig. 4 illustrates

photographically the floral organization of *Epilobium hirsutum,* as seen in transverse section of the bud just about to open. The illustration shows the floral envelopes

FIG. 4

and the stamens, together with the pistillary portion of the flower, the latter being somewhat displaced in the figure and cut through the region of the style. The long hairs characteristic of the calyx of this species have been trimmed off, for the purpose of facilitating photomechanical reproduction. As in the two illustrations above, the anther sacks are the most significant feature. Even with the low magnification employed for the purpose of illustrating the whole flower, the pollen grains in the loculaments of the anthers are easily discernible and present a striking contrast to those of *E. angustifolium,* in the respect that they are in groups of tetrads. Some of the groups are partially or wholly made up of individual grains without protoplasmic contents, which are smaller in size than the normal grains. Fig. 5 shows one of the anthers much more highly magnified. The anther walls, cavities and the pollen grains are now clearly distinguish-

able. Some of the grains are full size and present dark contents. Others are considerably smaller and are devoid of protoplasm. The latter are abortive or sterile grains. We have in fact before us a hybrid derivative of *E. hirsutum,* commonly found near ballast in New England and not unfrequently cultivated in gardens. Other species of *Epilobium* in the stricter sense of the generic appellation, show similarly abortive pollen development and the conclusion reached by old world systematists on the external

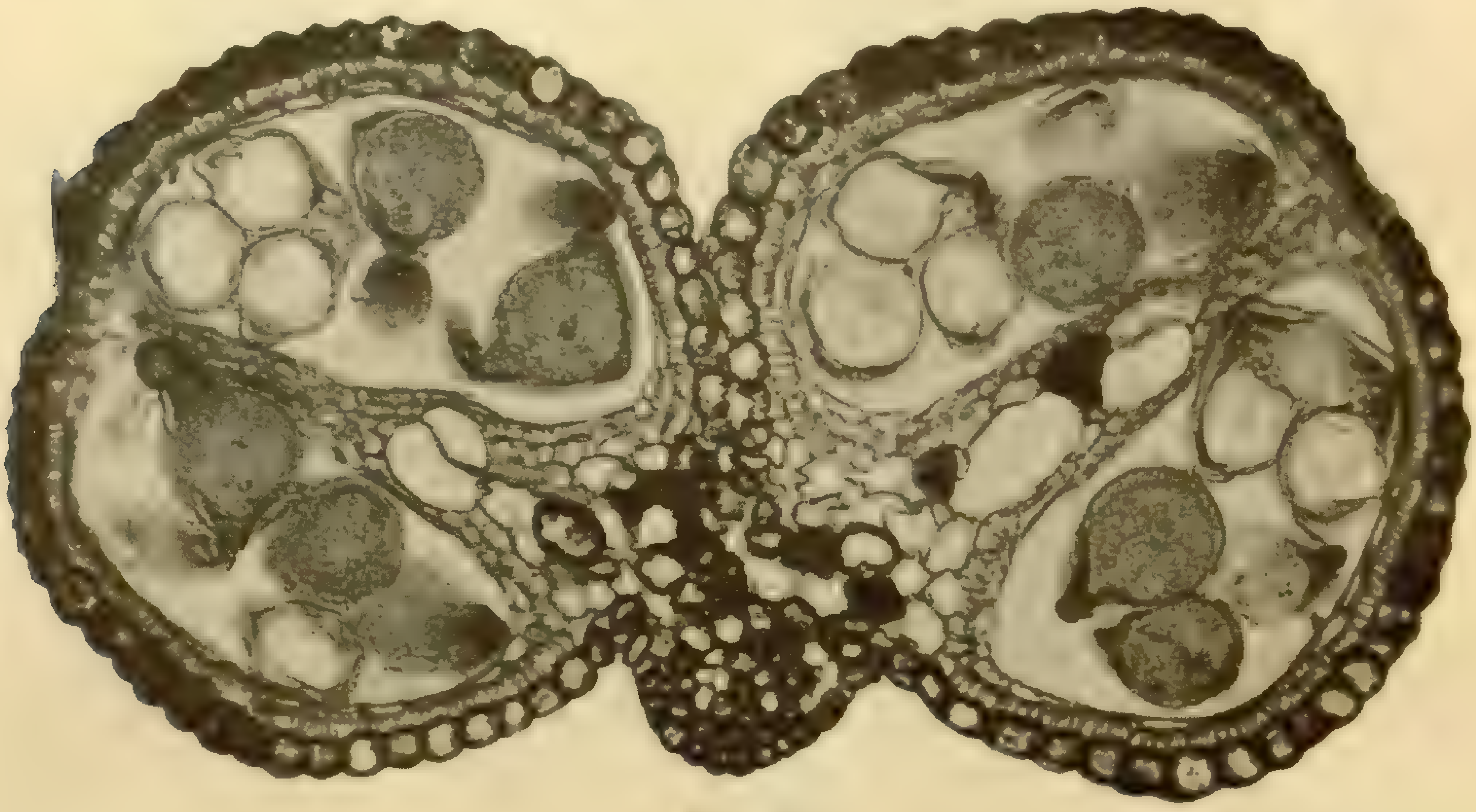


FIG. 5

characters, that hybridization is common among the species of *Epilobium* proper, is entirely confirmed by the study of the pollen. It need hardly be emphasized in this connection, that imperfect pollen development has been recognized for nearly a century by scientific plant breeders, as a criterion of hybrids.

The genus *Œnothera* may now be profitably considered. Fig. 6 presents a magnified view of a transverse section of a mature flower bud of one of the commonest of eastern species of *Œnothera,* namely *Œnothera biennis.* The floral envelopes are more voluminous than in the two genera illustrated above. Within are the stamens and in the center of the figure the style appears as a large rounded structure. Even with the low magnification employed, it is easy to discern that the contents of the anther sacks present a very different appearance from those of

Epilobium angustifolium. Many of the grains of pollen are light colored and devoid of the protoplasm which gives a dark appearance to the sound grains. Fig. 7 illus-

FIG. 6

trates a single stamen under a high degree of magnification. The characteristic layers of the wall of the anther sack, described comparatively and in detail in the classic memoir of Chatin, can readily be distinguished. Within lie the pollen grains. Clearly only a few of these are fully developed and possess normal protoplasmic contents. The greater number are shrivelled and empty. Judged from the generally accepted canon of the abnormalities of hybrids, *O. biennis* is of hybrid origin. This view of its nature is in harmony with its wide degree of inconstancy throughout its very extended range. This feature is doubtless responsible for the fact that the genus *Œnothera* is at the present time undergoing considerable elaboration, on the part of systematists. I have satisfied myself that the pollen peculiarities of *O. biennis* are uniformly present in specimens collected hundreds of miles apart, from the Province of Ontario, the shores of the Gulf of St.

Lawrence and the New England States. I have further examined a large number of species of *Œnothera* from various parts of the continent and in every instance have found a greater or smaller amount of abortive pollen as a characteristic feature of the anther contents. De Vries in his "Mutationstheorie" describes the abortive condition of about one third of the grains in *O. lamarckiana.* This feature has been seized upon with insight by Bateson, as indicating the hybrid origin of *O. lamarckiana.* It is extremely curious that its significance should have

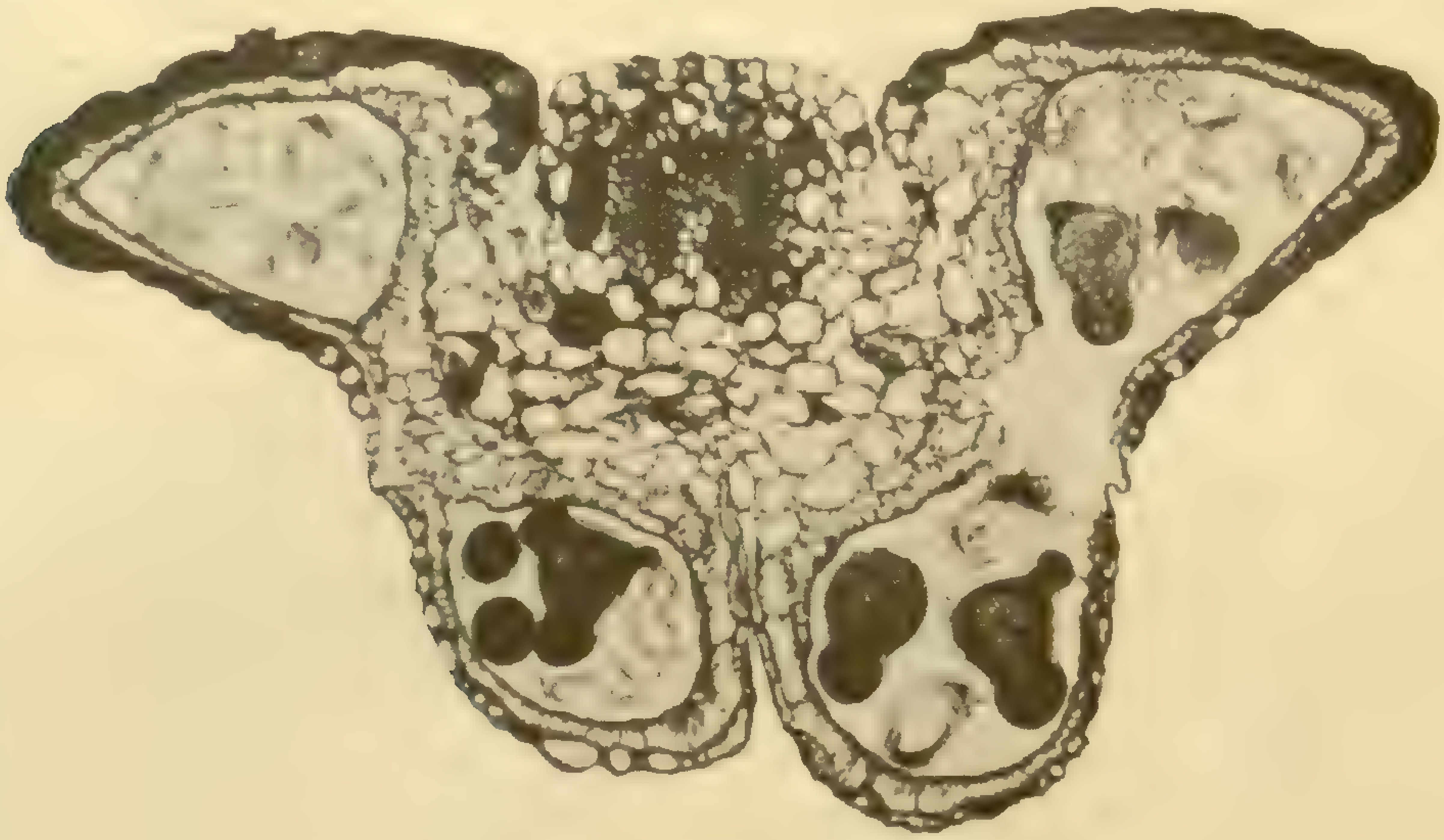


FIG. 7

escaped De Vries and his numerous disciples on this continent. Not only is *O. lamarckiana* itself characterized by a large proportion of abortive pollen but its so-called mutants are similarly characterized. In the feebler "elementary species" the pollen is often almost entirely abortive (*O. nanella*) and this is also generally the situation in *O. lata.* It should be further noted in this connection that if *O. lamarckiana* is of hybrid origin, the same statement must hold of the other species of *Œnothera,* since like this much-disputed one, they are similarly characterized, so far as they have been studied, by two correlated features, namely more or less abortive pollen and the peculiarity of throwing so-called mutants or "elementary species" in cultures. As a consequence of this condition,

it becomes more or less a superfluity to study any particular species of *Œnothera* from the genetical and morphological standpoint, since it is the genus as a whole which manifests the peculiar features, which have brought it so much into the foreground of biological controversy during the past decade. This is on the whole a satisfactory situation as it enables us to cut the perplexing gordian knot involving the controverted origin of *O. lamarckiana.* The mutation hypothesis of De Vries accordingly turns not upon the finding of new herbarium specimens which may throw light upon the origin of a particular species but upon the much larger question of the genetical status of the genus *Œnothera* as a whole. This question can be settled only by consideration of the Onagraceæ as a whole and of other families of the Angiosperms, which present similar reproductive peculiarities.

Before proceeding however to the discussion of the facts recorded above in their relation to the mutation hypothesis of De Vries, based on the conduct of *O. lamarckiana* in cultures, it will be necessary to make some brief reference to other studies carried on in the laboratories of plant morphology of Harvard University, which will be published elsewhere, either at the present time or at a later period. Obviously of great importance in the present connection is a comparison of the conditions of sporogeny found among the lower plants, the Bryophyta, the Pteridophyta and Gymnosperms, which are not characterized by enormous multiplication of species, with the sporogenic features of the Angiosperms in which the multiplication of species has run riot. Further comparison of liverworts, belonging to the Marchantiales, Anthosperms, manifesting similar sporogenic and specific peculiarities, is both pertinent and necessary, in the present connection.

It will be convenient to deal first summarily with the sporogenic conditions found in the lower forms of the Embryophyta from the Bryophyta to the Gymnosperms. In the present connection a considerable number of spe-

cies of liverworts, belonging to the Marchantiales, Anthocerotales and Jungermanniales, both acrogynous and anacrogynous have been examined with the general result that the only sterile cells present in the capsule cavities were the elaters. Infertile spores and hybridism both were conspicuous by their absence in the forms studied. The same statement *mutatis mutandis* holds for the true mosses. Some indication of spore abortion was detected in the extremely variable genus *Sphagnum*. It would seem that natural hybrids exist to some extent in this genus. Among the Pteridophyta both the Lycopsida and Pteropsida were studied. None of the numerous Lycopsid forms investigated showed signs of spore abortion or hybridism. Among the Pteropsida, the only well-known hybrids are found among what is probably the highest family, the Polypodiaceæ. There is a considerable literature upon hybrid ferns, in which references to spore abortion as an accompanying feature are common. No evidence of hybridism in the form of abortive spores was found in examples of the Marattiaceæ, Ophioglossaceæ, Osmundaceæ, Gleicheniaceæ, etc., were found, although a large amount of material was examined. Among the Gymnosperms, the Cycadales, Ginkgoales, Coniferales and Gnetales were examined. The Coniferales yielded only a single species of *Abies*, which showed evidence by the presence of abortive pollen grains of hybrid origin. The genus *Pinus* is very old and its species accordingly very distinct. Not the slightest evidence of hybridization was found here or in other numerous and widely distributed species of conifers, other than *Abies* mentioned above. This does not of course preclude the discovery of such conditions later. The writer has had the opportunity of examining the spores of a number of fossil forms from the Paleozoic and Mesozoic, still contained within the sporangia, and in no case were abortive spores recognized. The general conclusion can be drawn from the forms just considered that hybridism is rare among them and that

where it occurs it is accompanied by the phenomenon of spore abortion.

If we turn to the Angiosperms with their nearly one hundred and fifty thousand recognized species, we find that hybridism is very commonly recognized. It would take us much too far to discuss the situation here at any length. The consideration of a single important family must suffice. The one chosen, as being of particular significance in the present connection, is the Rosaceæ. We have had a recognition for many years past on the part of systematic botanists in this country and in Europe that hybridism is extremely common as a natural condition in certain genera of the Rosaceæ. The inference in such cases is generally based on the blended character of the hybrids themselves, which show to a large extent a combination of the characters of their parent species. Professor Brainerd has recently made some very interesting investigations in this direction in the case of American representatives of the Rosaceæ. The recognized hybrid forms in the Rosaceæ are usually characterized by a considerable degree of pollen sterility, unless the parents happen to be species not very remote in relationship. In addition to the recognized hybrids of the rosaceous species, the work carried on in the Harvard laboratories has revealed a large number of hidden hybrids or crypthybrids, which are quite constant in their characters and are recognized by systematists as good species, but differ from normal species in the fact that their reproductive cells are to a greater or less degree abortive. Species of this kind are extremely common among those rosaceous genera, which have become of economic importance, such as *Rubus*, *Rosa*, *Pyrus*, *Malus*, *Sorbus*, *Cratægus*, etc. Taking *Rosa* as an illustration, in addition to numerous recognized hybrids, there are many types recognized as good species, *e. g.*, *Rosa blanda*, in which the pollen is normally largely abortive, in still other species, frequently those which are isolated geographically, the pollen is quite sound, *e. g.*, *Rosa rugosa* of Japan. The latter type of species must be

regarded as a species in the strict sense, while those of the type of *Rosa blanda,* in which abortive pollen similar to that characteristic of forms clearly recognized as hybrids, is present, are hidden hybrids. It follows that in *Rosa* (or practically any of the other rosaceous genera cited above), there are three types of individuals, namely good species, hidden hybrids and open hybrids. The middle condition is extremely common among the Angiosperms and is of the greatest importance in connection with clear views in regard to the origin of species. Obviously constant or relatively constant hybrids can not rank with pure species, such as are characteristic for example of the Gymnosperms, in discussions in regard to the origin of species by mutation or otherwise. The conduct of such forms is conditioned to a greater or less extent by their mixed blood. We may appropriately designate obvious hybrids as phenhybrids and those hybrids which are recognizable as such by their internal morphological characters as crypthybrids. Crypthybrids will probably when studied more extensively in cultures by the geneticist, give evidence of their hybrid origin in cultures. There can be no doubt that many of the recognized species of the Angiosperms are in reality crypthybrids. The enormous multiplication of species in this great group of plants is in all probability largely related to hybrid crossing. It is of the utmost importance however to keep clearly in mind that such hybrid species or crypthybrids are not at all in the position of true species from the evolutionary standpoint and that conclusions derived from their study can not be applied without large reserves, to the question of the origin of species in the strict sense. The species of *Pinus,* so far as we have any evidence, since the main types are known to have existed well back into the Mesozoic, in all probability illustrate the origin of species somewhat along the lines of the Darwinian hypothesis. On the other hand the species of *Rosa* present obviously an entirely different problem in evolution and the necessity of making distinctions if we are to reach any definite bio-

logical goal is very clear. A great deal of the pessimism which at the present time is sending too many biologists after strange gods in other scientific shrines is doubtless to be traced to the failure to make this distinction. It may not be possible to make the distinction in all cases even among the higher plants; but it certainly will be necessary to realize its significance. Probably plants will in regard to this possibility enjoy in this respect, as in so many others, an advantage over animals in the studies of the experimental evolutionist.

We may now consider with advantage the status of the species of the genus *Œnothera.* The pollen sterility which characterized them all to a greater or less degree is indisputable evidence of their probable hybrid origin. The general situation in regard to the criteria of hybridism in plants has been recognized for nearly a hundred years. It has been made clear by Bateson in regard to *Œnothera lamarckiana.* The observations chronicled here appear to make it obvious that all the species of *Œnothera* are in the same boat genetically, that is that they are all of hybrid origin. They likewise probably will all be found to "mutate" just as *O. lamarckiana, O. biennis,* etc., are already known to do. It may appear later that there are certain species which have escaped, through geographical isolation or other causes, the mingling of blood, which is certainly characteristic of the *Œnotheras* of the Eastern United States. So far as we know them at present, the species of *Œnothera* are obviously in the same position as such species as *Rosa blanda,* that is they are crypthybrids. Doubtless the peculiarities of *O. lamarckiana, O. biennis,* etc., can be more clearly explained in the present condition of our knowledge as the result of hybrid origin than in any other way. It follows that the doctrine of mutation so far as it depends for its support upon the *Œnotheras* is in a discredited condition, as an explanation, in any proper sense of the term, of the origin of species.

Conclusions

1. The Onagraceæ are largely characterized by hybrid contamination in nature.

2. This statement holds with particular force for *Œnothera lamarckiana* and other species of the genus *Œnothera,* which have served as the most important basis of the mutation hypothesis of De Vries.

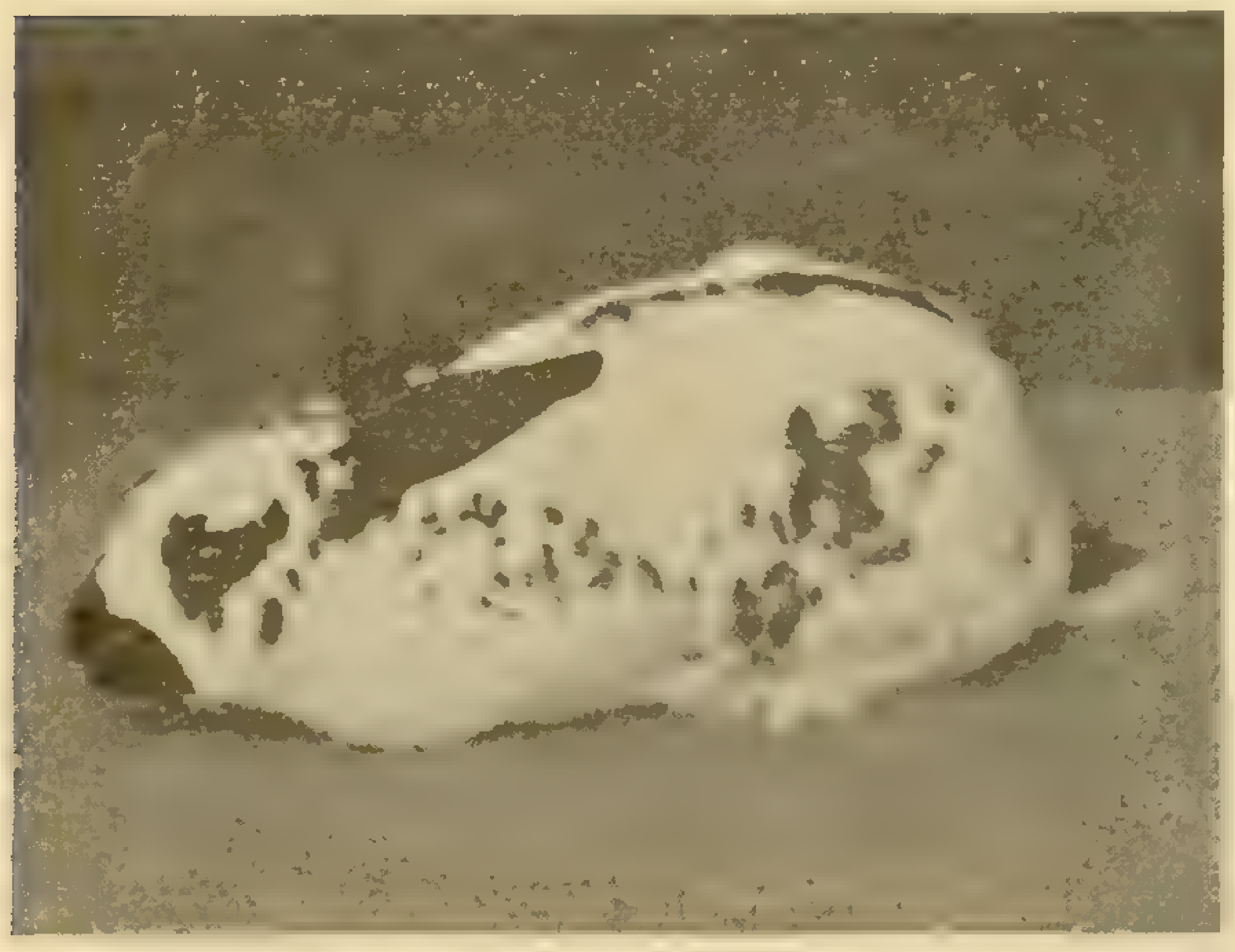
3. Constant hybrids or crypthybrids are of very common occurrence among the Angiosperms and have been illustrated in the present article by reference to the genetical conditions occurring in certain Rosaceæ.

4. The species of *Œnothera* are to a large extent, if not wholly, crypthybrids.

5. The objection raised by Bateson to the genetical purity of *Œnothera lamarckiana* is confirmed and is extended to the Onagraceæ in a general way, as well as to other species of *Œnothera.*

6. Hybridism is the best explanation yet put forward of the peculiar conduct of *Œnothera lamarckiana,* as well as other species of the genus in cultures.

7. The mutation hypothesis of De Vries, so far as it is supported by the case of *Œnothera lamarckiana,* is invalidated.



FIGS. 1 AND 2 AT TOP; FIGS. 3 AND 4 IN THE MIDDLE; FIGS. 5 AND 6 AT BOTTOM. Figs. 1-4, Photographs of Four English Rabbits forming a Graded Series in Amount of Colored Fur. These were adopted as our standard Grades 1-4, in terms of which all the rabbits described in these experiments have been classified. Fig. 5, Rabbit ♂21*A*, Father of the Series I Young. Fig. 6, Rabbit ♂40*A*, Father of the Series II Young.

THE ENGLISH RABBIT AND THE QUESTION OF MENDELIAN UNIT-CHARACTER CONSTANCY

W. E. CASTLE AND PHILIP B. HADLEY[1]

WHATEVER the theoretical importance of Mendel's law, its practical utility depends largely upon the purity of the gametes. If Mendelian unit-characters can through hybridization be recombined in desirable ways *without essential modification* during the process, Mendel's law is evidently a distinct acquisition to the practical breeder. Nevertheless, if crossing is likely to produce considerable changes in the characters which it is desired to combine in a new race, it is evident that Mendelian crosses must be used judiciously and with caution by the practical breeder.

Considerations such as these have led the senior author for several years to concentrate his studies of genetic problems upon the question of gametic purity. As a crucial experiment he conceived the plan of deriving an entire race of animals, not from a single *pair* of ancestors, but from a single *gamete,* so far as concerns a particular unit-character. It was thought that in a race so derived, if the principle of gametic purity holds, there should be no variation whatever in the particular unit-character concerned.

Color patterns of mammals seemed especially well adapted for such studies, since they are early differentiated and clearly Mendelize in crosses. The so-called "English" piebald rabbit presents an especially fine example of such a color pattern. The figures give a good idea of this striking pattern in which white and colored areas are interspersed much as in the "coach-

[1] Joint publication of the Laboratory of Genetics of the Bussey Institution, Harvard University, and of the Agricultural Experiment Station of the Rhode Island State College (Contribution 211).

dog." It would be a distinct gain to breeders if they could reduce the variation in details of the English pattern so that "prize-winners" could be bred without the production of so many "wasters," which depart in essential points from the standard pattern adopted for the breed. This was an additional reason for undertaking work with the English rabbit.

The first standard-bred English rabbits which the senior author had under observation, when mated *inter se,* produced young of three sorts. About half the young were fairly good "standard" English extensively marked with colored spots (see Fig. 3). About one fourth were much whiter than the standard demands, their spots being fewer and smaller (see Fig. 1). And the remaining fourth were without spots, that is, were self colored. This last class was found to be recessive and not to produce English offspring, if mated *inter se.*

The *whiter-than-standard* English proved to be homozygous for the pattern, the "standard" English being heterozygous and breeding like their parents.

From these observations it was clear (1) that the English pattern is a Mendelian dominant and (2) that the breeding of English rabbits resembles that of blue Andalusian fowls. For the standard-bred animal is a heterozygote in the production of which there is bound to be a constant production of "wasters" unless either the standard is changed or the homozygote can be changed to conform with the standard, producing an animal with more color. In the latter case homozygotes could be bred with each other and wasters eliminated. The question whether the pattern can be changed becomes therefore one of practical as well as theoretical interest.

In making crosses of English with other breeds of rabbits, there was found to be considerable variation among the heterozygous English produced, some being much whiter than others, *i. e.,* having less extensive colored spots. Plus (dark) and minus (light) selections were made to see to what extent the pattern was capable

of modification. These selection experiments are still in progress, but will be reported upon at another time.

The single-gamete experiment, with which this report will deal, was placed in the hands of the junior author, who has carried it out at the Rhode Island Agricultural Experiment Station.

As foundation stock for the experiment a single *heterozygous* English rabbit of standard character (grade 2, Fig. 5) was selected. To mate with him, it was desired to obtain a distinct breed of rabbits, free from the English pattern, and as pure (uniform) in all respects as possible. For this purpose the "Belgian hare" was chosen. A buck and two does obtained from Mr. G. W. Felton, Cliftondale, Mass., were found to breed very true. From them was bred a stock of does very uniform in character, twelve of which, together with one of the parents (2*A*), were mated with the selected English buck which we may henceforth call by his record number ♂21*A*. The young thus produced will be called "Series I" offspring. About half of them were self (non-English), the remainder (187 in number) were English.[2] The latter, although all undoubtedly heterozygous, varied in whiteness from grade 1 to grade 4 (Figs. 1–4), the *modal* or commonest condition being about the same as that of the father (grade 2). The distribution of the young in relation to our grades is shown in Table I. Statistical treatment of the table gives the *average grade* of the young as 2.43, that is somewhat darker than the father. Inspection of the table shows that more than half of the young are *darker* than the father, which supports in a general way the statistical average grade. If we consider separately the average grade of the young produced by each

[2] The total number of young obtained from ♂21*A*, when mated with Belgian hare does, has been to the time of writing 436. The English young now number 210, the non-English (self) number 226. For Series II matings presently to be described the corresponding numbers of young are: English, 219, non-English 196, total 415. For Series I and II combined the numbers are: English 429, non-English 422, total 851. This is unmistakably a 1:1 Mendelian ratio.

mother, we find that it ranges from 2.15 in the case of ♀18*F*, which had 5 English young, to 2.79 in the case of ♀16*D*, which had 14 English young. The average number of English young to a mother is 14.4.

After this series of matings had been completed, a second series was begun in which the same 13 females were mated with one of the darkest bucks produced in the Series I matings (a son of ♀16*E*). The selected buck was ♂40*A* (Fig. 6), grade 3.75, considerably darker than his father (Fig. 5). This series of matings produced 189 English young, together with a like number of self (non-English) young. The grade distribution of the English young is shown in Table I, Series II. All of the 13 mothers except one (♀16*F*) produced darker offspring in the Series II than in the Series I matings. The lowest average grade was shown by the young of ♀17*G*, viz., 2.44. For Series I matings the lowest average was 2.15. The highest average grade in the Series II matings was given by the young of ♀16*E*, viz., 3.50. For Series I matings the highest average was 2.78. Consequently, both maximum and minimum averages were higher in the Series II than in the Series I matings. The grand average of all the 189 Series II offspring was 2.92 as compared with 2.43, the average grade of the Series I young. Their modal grade is 3.25. The modal grade for Series I was 2.00. Since the mothers were identical in both series, the difference in the young can be attributed only to the difference in the fathers. The male used in the Series II matings differed genetically as well as somatically from his father, who sired the Series I young. Not only was he darker, but he also produced darker English young. Yet the father contained only a single dose (one gamete) of English pattern and the son derived his English pattern exclusively from this same source. Hence the English unit-character had changed quantitatively in transmission from father to son. This seems to us conclusive evidence against the idea of unit-character constancy, or "gametic purity." If unit-characters are not constant, selection

reacquires much of the importance which it was regarded as possessing in Darwin's scheme of evolution, an importance which many have recently denied to it.

TABLE I

SHOWING THE DISTRIBUTION OF GRADES OF OFFSPRING IN THE FIRST AND SECOND SERIES OF MATINGS FOR EACH INDIVIDUAL MOTHER

Mother	Series	Grades of Young													Totals		Average
		1.00	1.25	1.50	1.75	2.00	2.25	2.50	2.75	3.00	3.25	3.50	3.75	4.00	Ser. I	Ser. II	
16*A*	I	...	...	...	...	1	2	2	...	...	...	...	...	...	5	.....	2.30
	II	...	...	...	...	3	1	1	2	1	7	3	3	...	.....	21	3.03
16*B*	I	...	1	...	1	3	1	1	1	2	...	...	1	...	11	.....	2.39
	II	...	...	1	1	2	1	...	1	2	1	3	...	...	.....	12	2.67
16*D*	I	...	...	...	...	2	5	...	...	1	2	3	1	...	14	.....	2.79
	II	...	...	...	...	...	...	...	3	1	6	...	2	...	.....	12	3.19
16*E*	I	...	...	2	...	5	...	2	1	1	...	...	1	...	12	.....	2.29
	II	...	...	...	...	...	...	...	...	...	1	3	1	...	.....	5	3.50
16*F*	I	1	...	...	...	2	1	5	3	2	...	3	...	...	17	.....	2.62
	II	...	...	...	1	2	1	3	3	...	...	1	...	...	.....	11	2.48
16*G*	I	...	1	2	...	2	3	1	2	3	1	...	...	...	15	.....	2.35
	II	...	...	...	...	...	1	2	2	3	1	2	1	1	.....	13	3.06
16*H*	I	...	...	1	...	3	3	1	2	2	2	1	2	1	18	.....	2.76
	II	...	...	...	...	3	...	2	1	1	8	2	2	1	.....	20	3.06
17*E*	I	...	1	1	3	4	3	3	1	1	2	2	...	...	21	.....	2.36
	II	...	...	...	...	1	2	5	5	3	4	4	2	1	.....	27	2.97
17*G*	I	...	...	1	9	6	3	3	2	1	2	1	1	...	29	.....	2.27
	II	...	1	1	...	1	1	1	1	1	1	1	...	...	.....	9	2.44
18*D*	I	...	1	...	...	3	3	3	...	1	2	2	...	...	15	.....	2.53
	II	...	...	...	...	2	1	2	3	1	2	5	...	...	.....	16	2.91
18*F*	I	...	...	...	3	1	...	...	...	...	...	1	...	...	5	.....	2.15
	II	...	...	...	1	1	2	...	4	2	1	1	2	2	.....	16	2.97
18*H*	I	...	...	1	1	...	3	1	1	3	...	...	...	...	10	.....	2.43
	II	...	...	...	1	...	3	3	4	2	3	...	1	2	.....	19	2.87
2*A*	I	...	1	2	1	1	4	2	3	1	...	...	...	...	15	.....	2.22
	II	...	...	...	1	2	...	...	...	1	2	2	...	...	.....	8	2.78
Totals	I	1	5	10	18	33	31	24	16	18	11	13	6	1	187	.....	2.43
	II	...	1	2	5	17	13	19	29	18	37	27	14	7	.....	189	2.92

The question whether an imaginary "unit-factor" for English pattern has or has not changed in correlation with the visibly changed English unit-character is not here discussed. We recognize that it has an academic interest, which, however, scarcely affects the practical question whether the visible Mendelizing characters of animals are subject to change through crossing or through selection or both.

CONTRIBUTIONS FROM THE ZOOLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOOLOGY AT HARVARD COLLEGE, NO. 256.

ON THE NUMBER OF RAYS IN ASTERIAS TENUISPINA LAMK. AT BERMUDA[1]

BY W. J. CROZIER

HARVARD UNIVERSITY

I. It was suggested by Clark (1901) that the average number of rays borne by *Asterias tenuispina* was different for separate localities in Bermuda. He examined forty specimens of this species—eleven from Coney Island and twenty-nine from Harrington Sound; in the first set the average number of rays was 5.91 (I find the mode to be 6), in the second set 6.93 (with a mode of 7). If this condition really obtains, it would be an exceedingly interesting matter to determine the factors responsible for this sort of difference. I have therefore examined a number of *Asterias* (312 in all) from several localities in the Bermudas, namely: Agar's Island, Spanish Point, Hawkins Island, Ely's Harbor, Hungry Bay, Harrington Sound and Coney Island; the first four are situated on the periphery of Great Sound, the others at widely removed points on the north and south shores. For the identification of these places, references may be made to the maps published by Mark (1905).

These observations were made at the Bermuda Biological Station, mostly during the summer of 1914.

II. The first lot of starfishes was collected in the immediate vicinity of Agar's Island in 1913. The number of rays varied from 2 to 9; the frequency distribution is given in Fig. 1. The modal number of rays is clearly 7. In 1914 a collection of *Asterias* from this place gave the ray frequency distribution shown in Fig. 2, where the modal number of rays is again 7. Collections, during 1914, at the other stations named gave the following ray frequency counts:

[1] Contributions from the Bermuda Biological Station for Research, No. 35.

Station and Year	No. of Specimens	Modal Ray Number	See Figure
Agar's Isl., 1913	43	7	1
Agar's Isl., 1914	62	7	2
Spanish Point, 1914	33	7	3
Hawkins Isl., 1914	39	7	4
Ely's Harbor, 1914	36	7	5
Hungry Bay, 1914	41	7	6
Coney Island, 1914	20	7	7
Harrington Sound, 1914	38	7	8

The modal number of rays is in each case 7. This is true for the same locality in two successive years, for near-by localities and for places widely enough separated

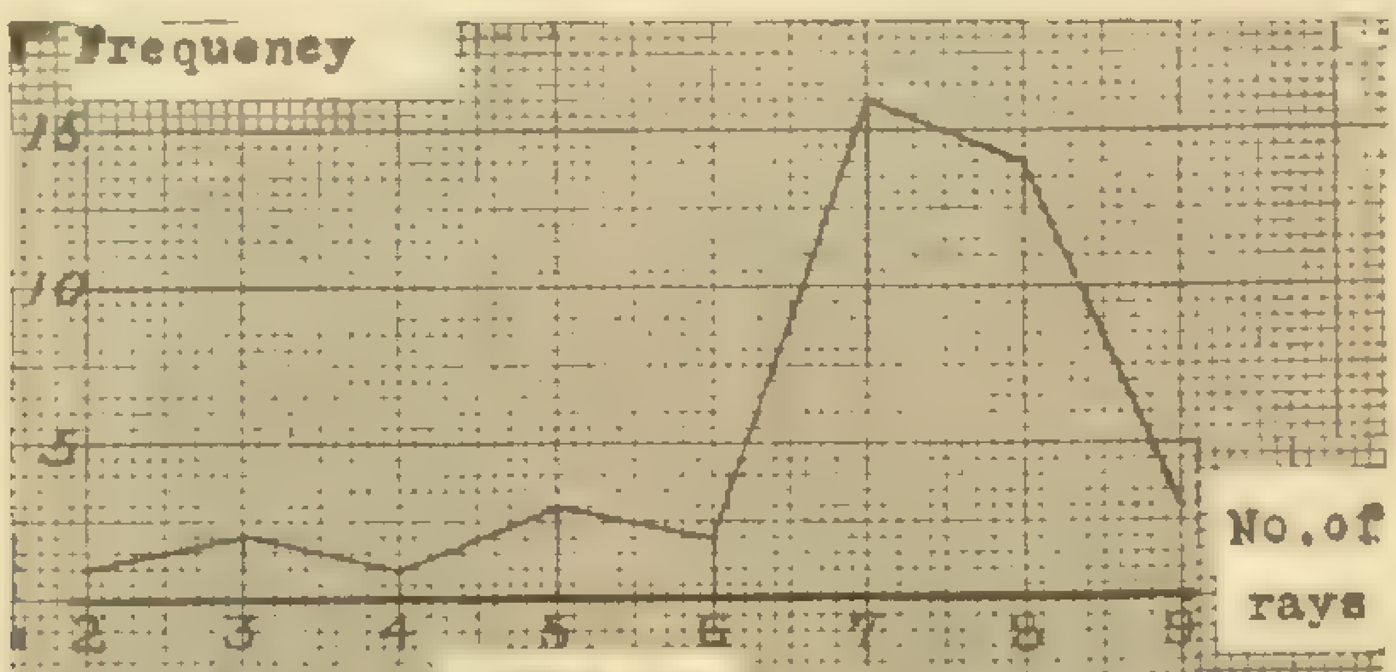


FIG. I
Agar's Island - 1913

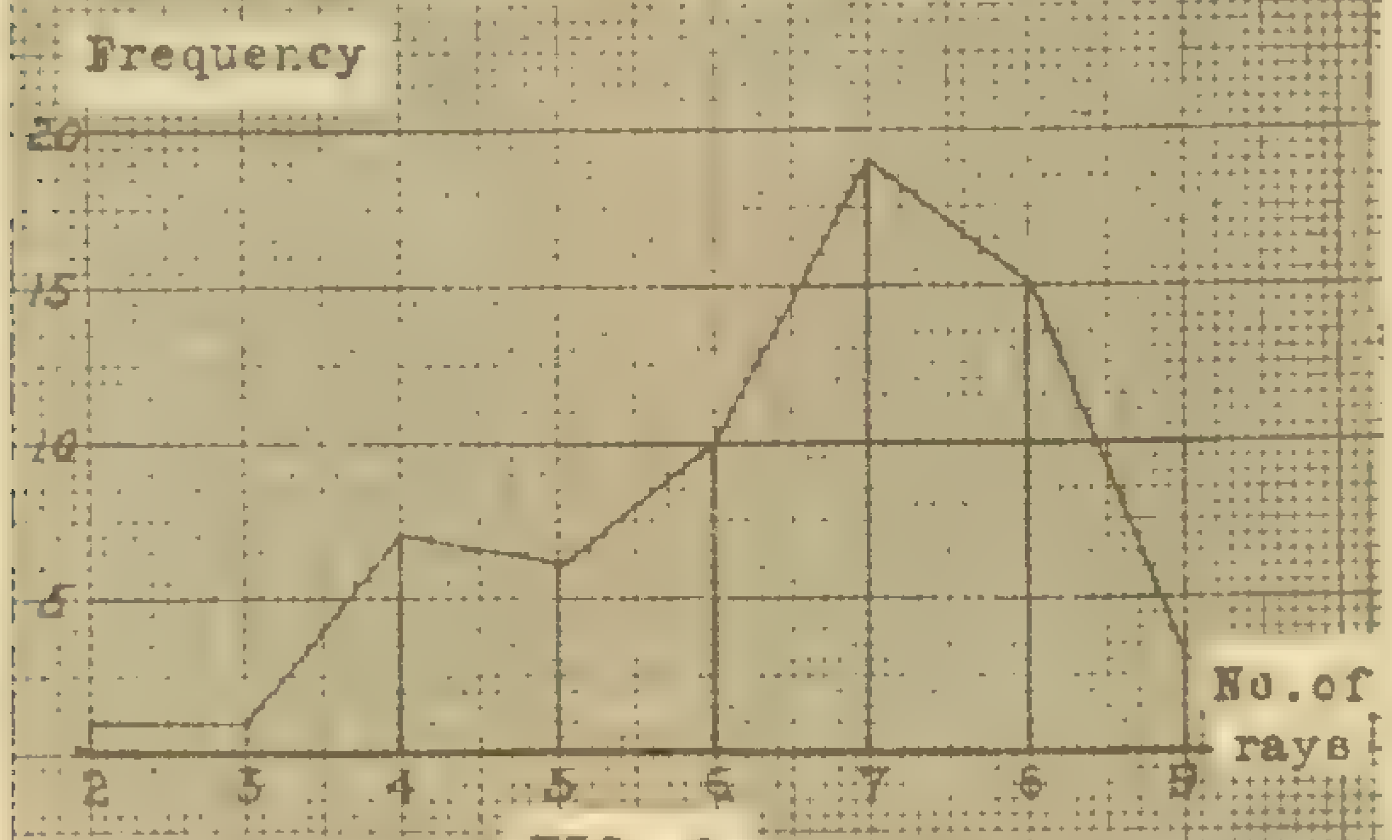


FIG. 2
Agar's Island - 1914.

to yield critical data relative to the suggestion which prompted this inquiry. For the total population examined the ray frequency distribution, which of course gives a mode of 7 rays, is plotted in Fig. 3. It is to be noted further that according to Ludwig (1897, p. 345) the most common number of rays in *A. tenuispina* from the Mediterranean is also 7.

III. It has been observed by every one who has studied *A. tenuispina* that in most of the individuals the rays occur in two groups, those of one group being longer than those in the other, though within each group the rays are of about the same length. This condition is evident in 259 (83.6 per cent.) of my specimens. There is general

agreement (cf. Ludwig, 1897, and Ritter and Crocker, 1900), that in some cases, if not in all, "this disparity in size is due to the regeneration of halves of automatically bisected animals." My observations fully confirm this. I have witnessed, as did Ludwig, several cases of spon-

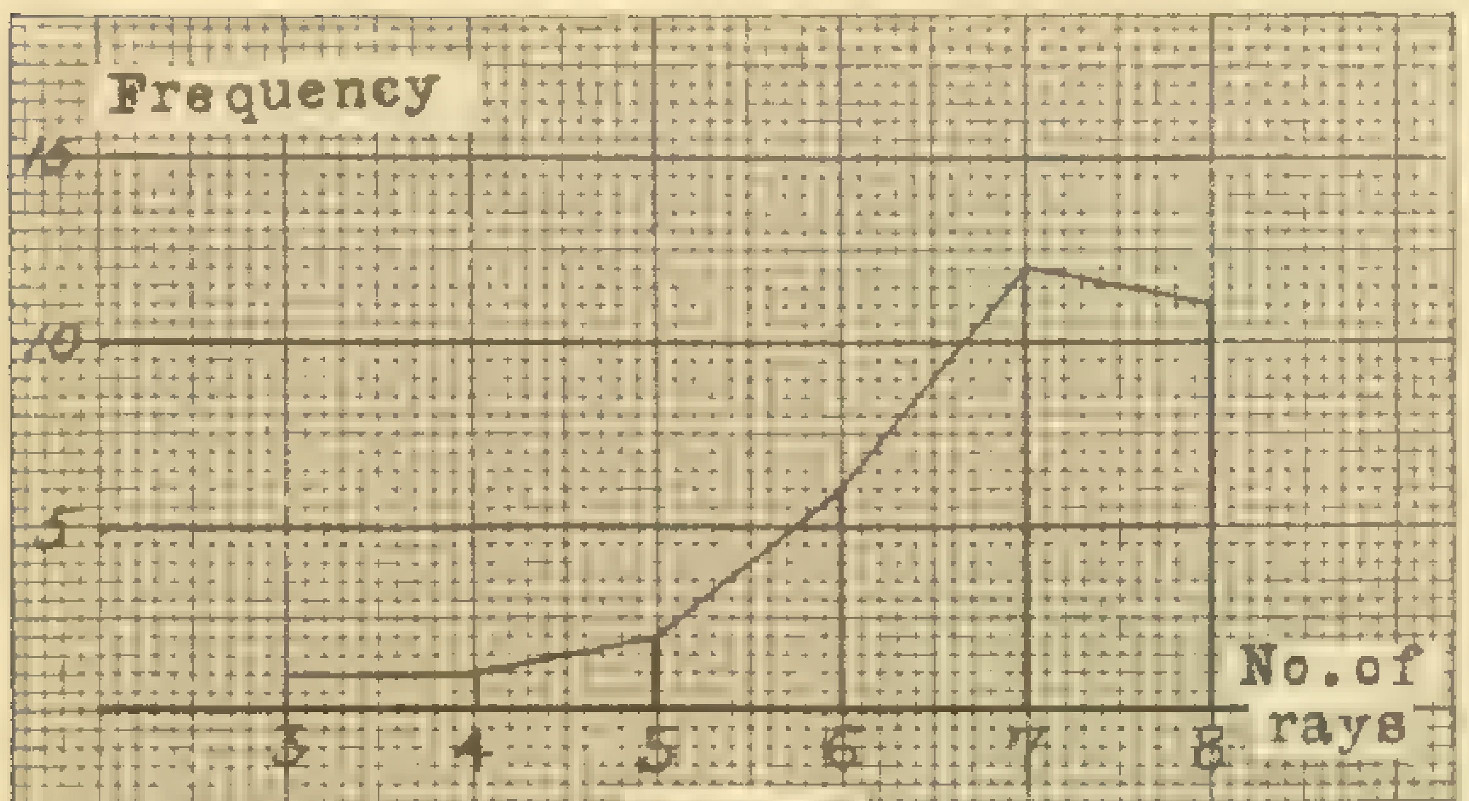


FIG. 3
Spanish Point

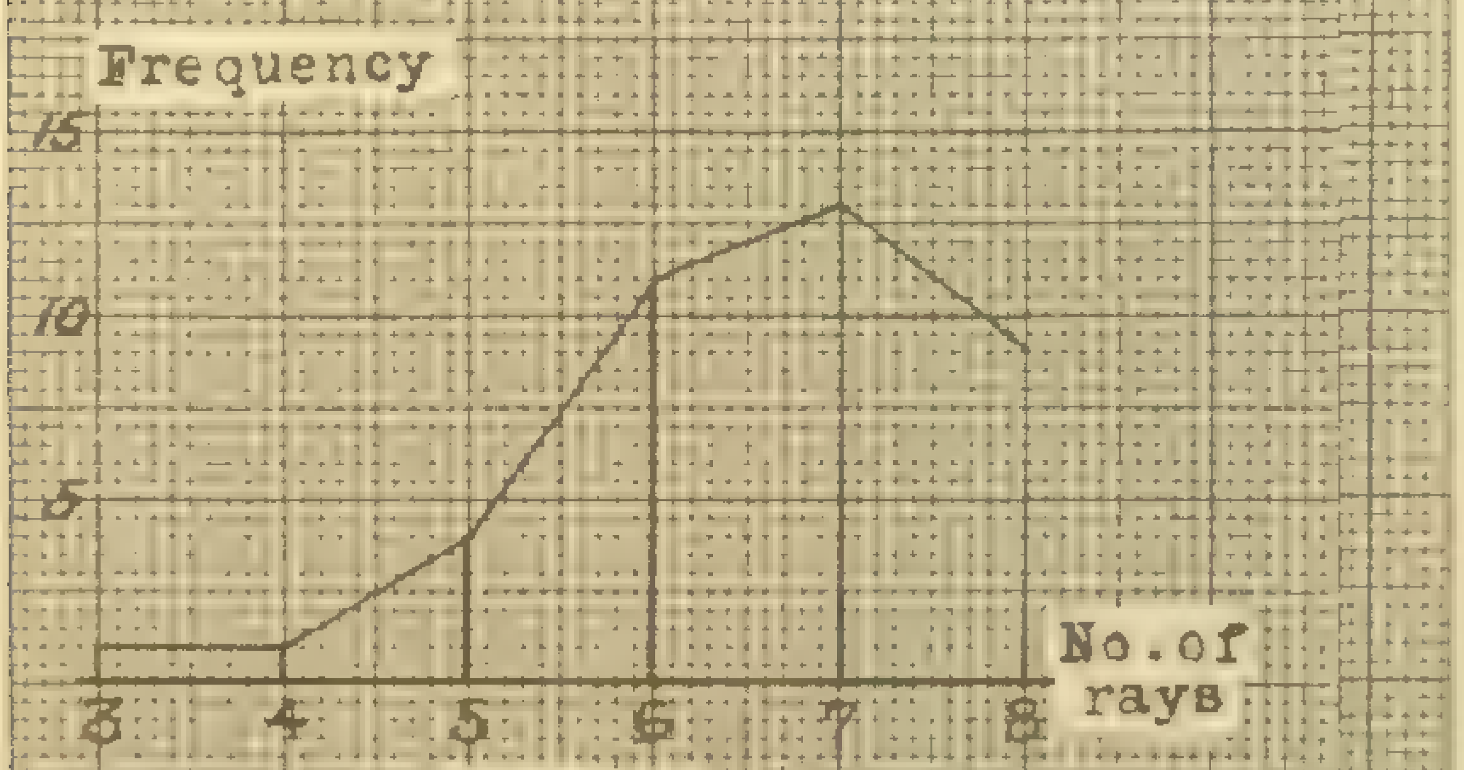


FIG. 4
Hawkins Island

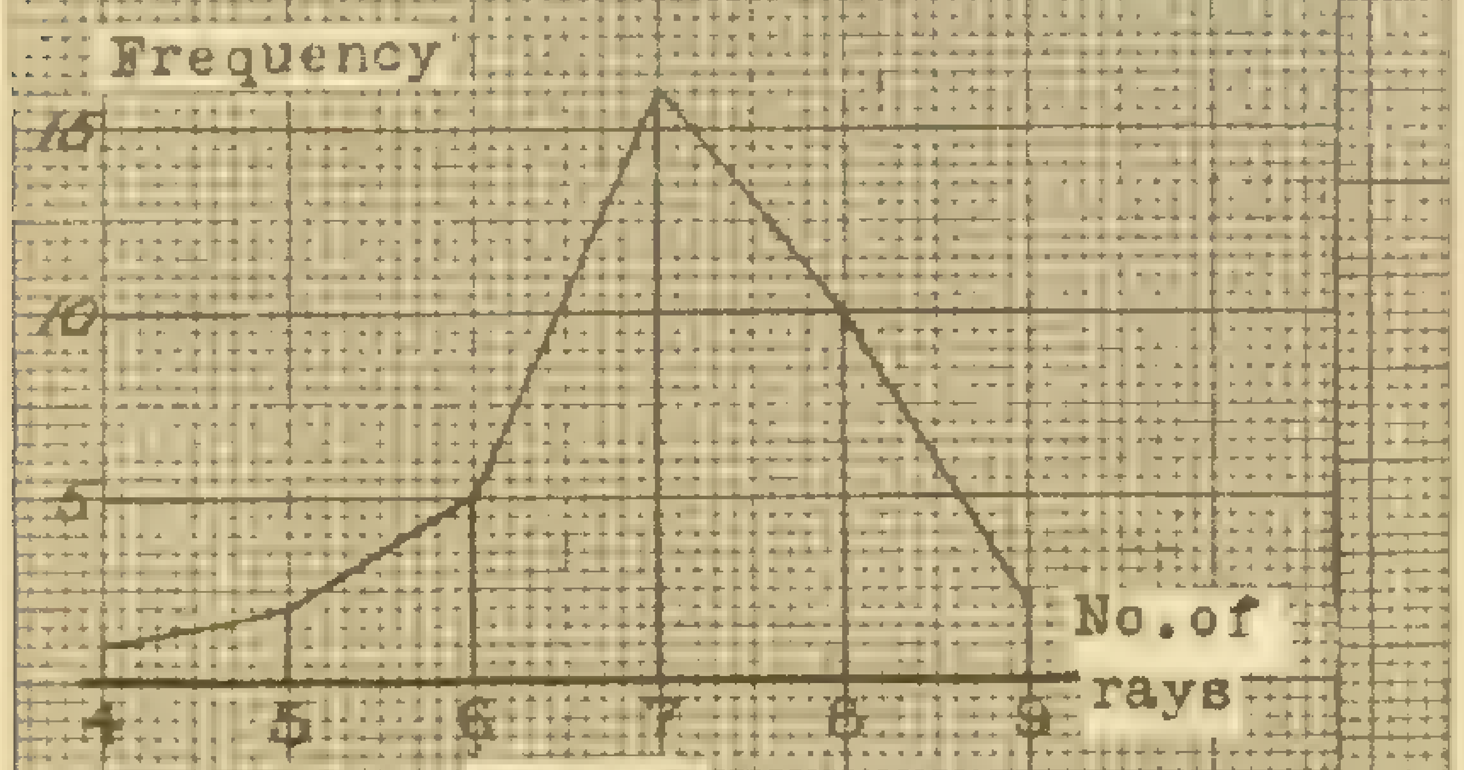


FIG. 5
Ely's Harber

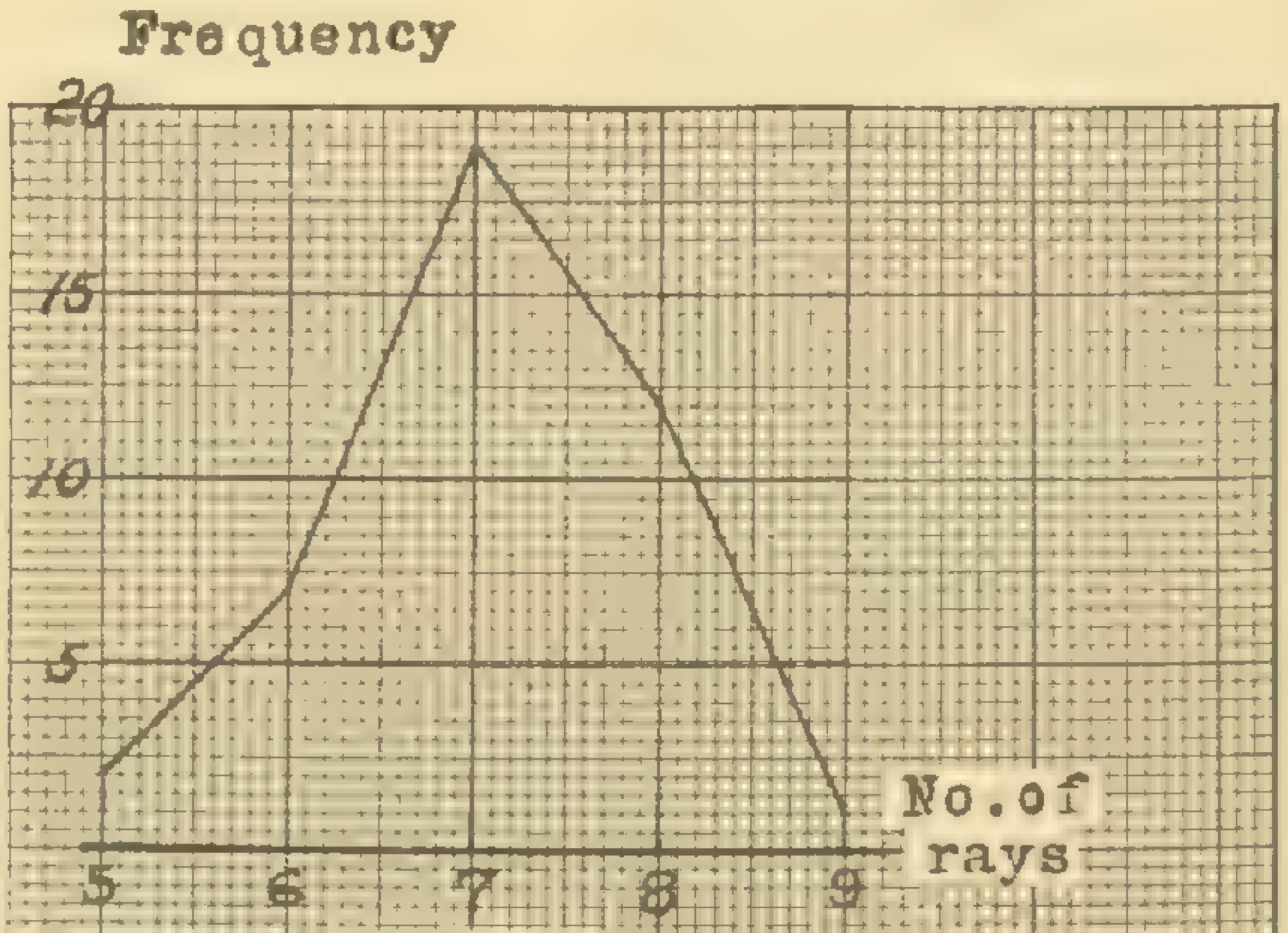


FIG. 6
Hungry Bay

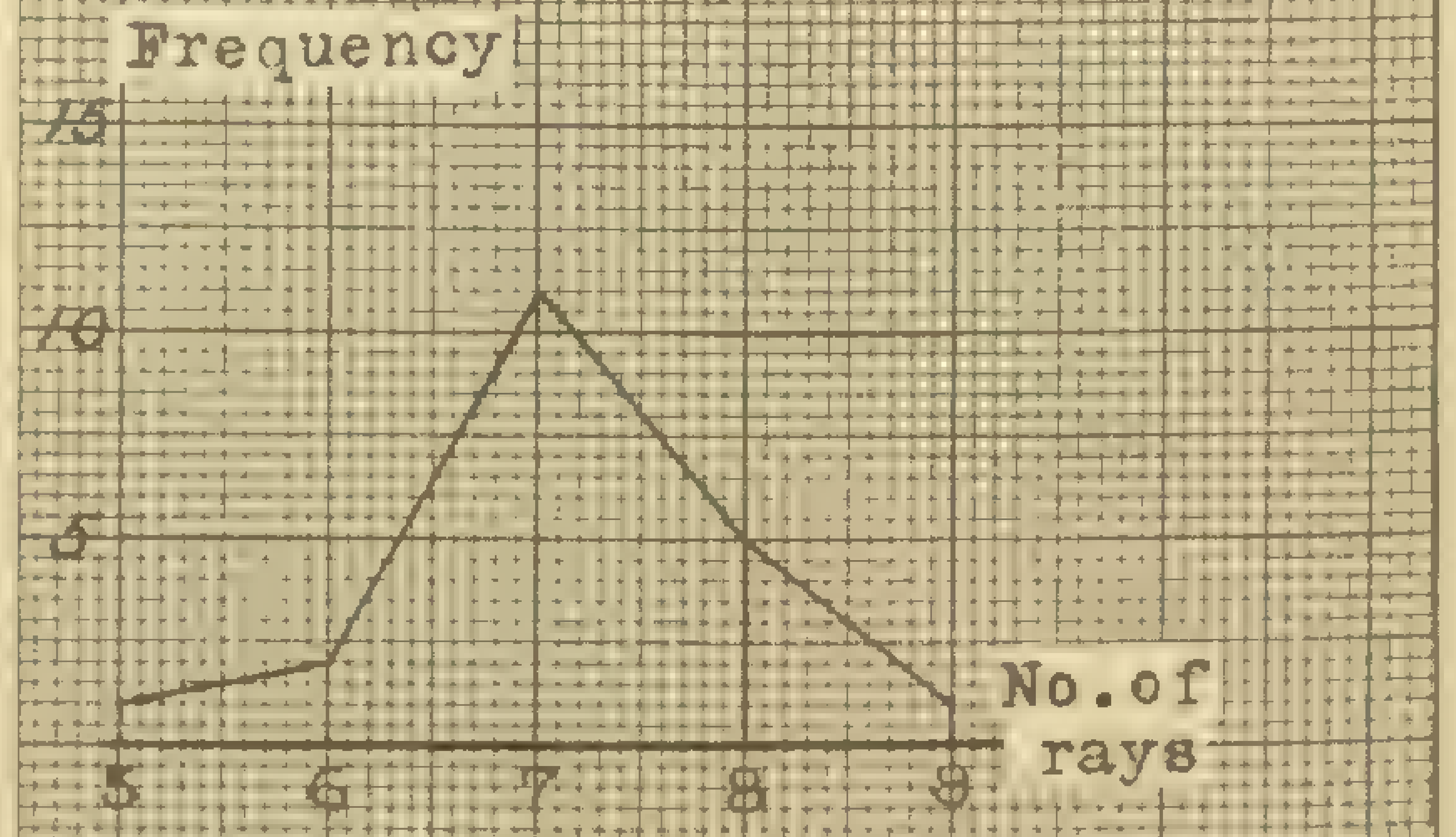


FIG. 7
Coney Island

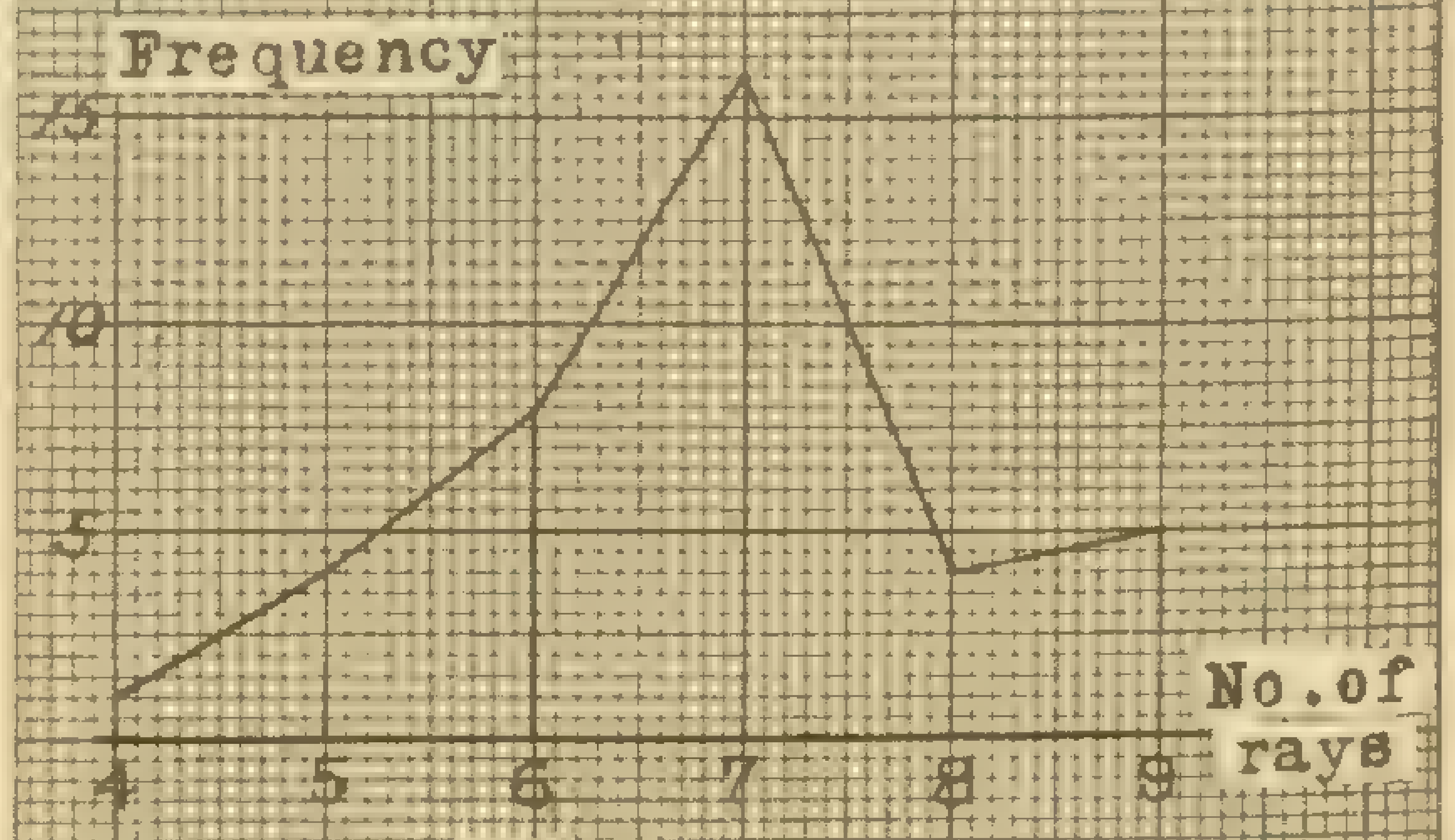


FIG. 8
Harrington Sound

taneous self-division in the laboratory. The casting off of one or more rays may at any time be induced by holding or injuring one or several rays, or by the stimulation of a ray with dilute acid applied with a pipette. The autotomy of a single ray takes place very much as described by King (1898) for *Asterias vulgaris;* the existence of a "breaking joint" in the region of the fifth ambulacral

ossicle is shown by the fact that even in preserved material the rays part very easily in that region.

The relative abundance of cases in which there are evident two groups of rays of different length indicates that, as in *Linckia* (Clark, 1913), autotomous division is a normal method of asexual reproduction.

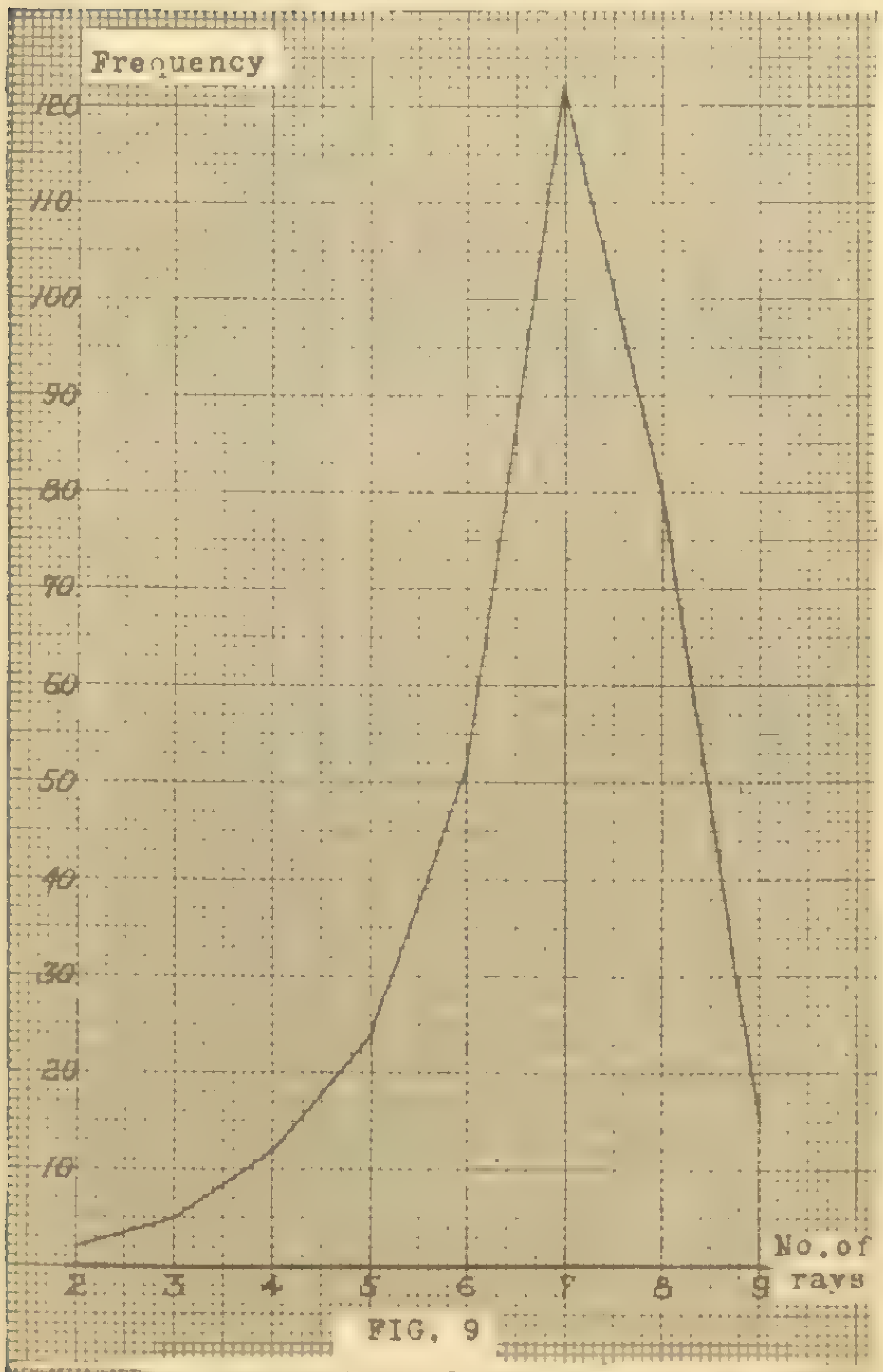


FIG. 9
Distribution of ray frequencies in the total population.

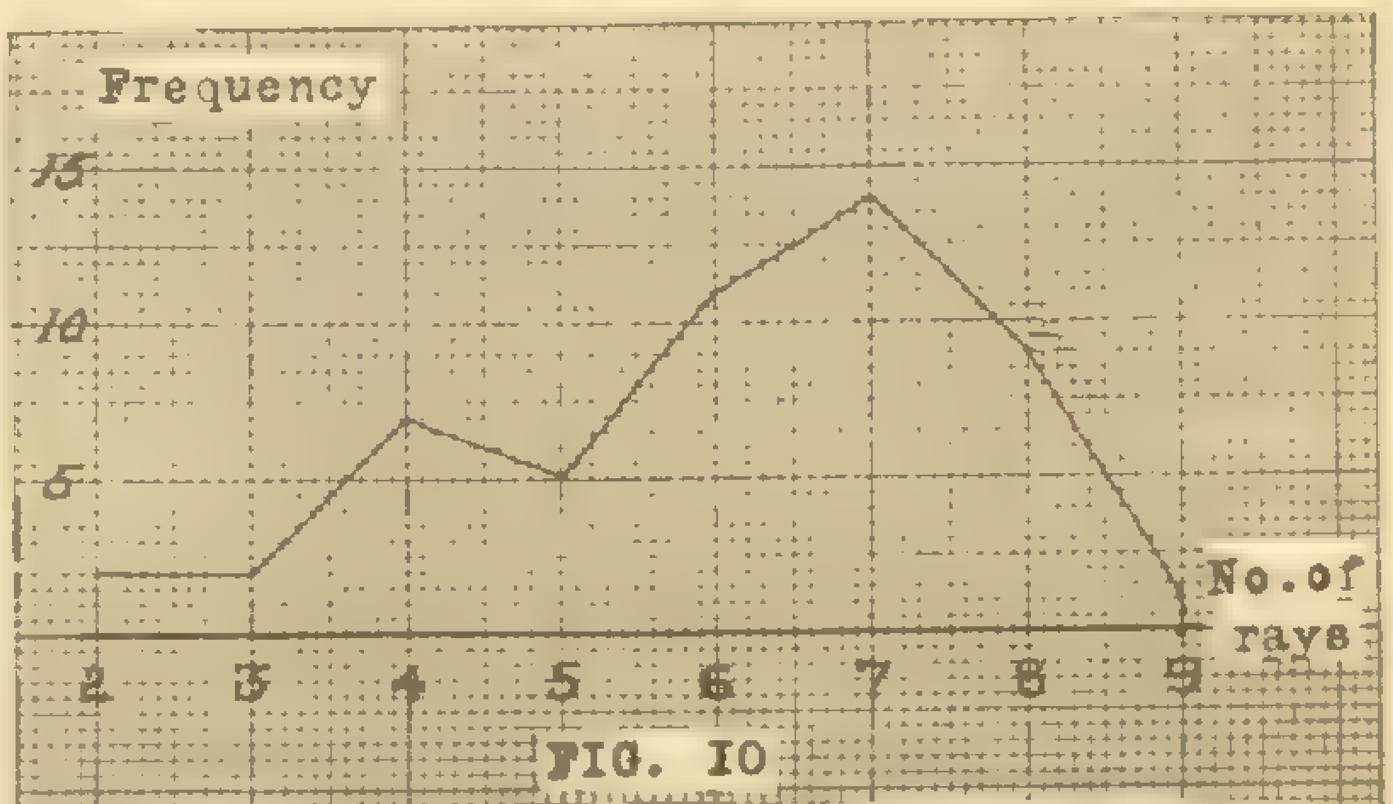


FIG. 10
Distribution of ray frequencies among individuals with all rays of equal or nearly equal length

SHORT RAYS \ LONG RAYS	1	2	3	4	5	6	7	
1			1	1	4	4	2	12
2			4	2	4			10
3		1	16	18	4			39
4		6	24	28	2			60
5		3	12	4				19
6	1	2						3
	1	12	57	53	4	4	2	143

FIG. 11
Relation of long to short rays

IV. The numerical relations of the old rays to the regenerating ones, and the topographical arrangement of the latter, yield evidence relative to certain questions in the physiology of regeneration.

(*a*) It is to be observed that the regeneration in question has taken place apart from experimental control; therefore information as to the number of rays usually present just before *Asterias* undergoes self-division must be deduced from the data at hand. The modal-ray frequency for specimens with rays of very nearly equal length is 7 (Fig. 10), but it is a question whether this appearance of equality in ray length may not be due to a

variety of conditions, especially the rapid growth of regenerated rays. The regenerating rays of *Linckia* (Clark, 1913; Monks, 1904) and the newly formed rays of multiradiate types (Ritter and Crocker, 1900) grow more rapidly than the old ones and soon reach the dimensions of the latter; this is also indicated in my series. But the correlation of the number of long with the number of short rays (using only those cases in which the two groups were clearly distinct) makes it evident (Fig. 11) that the condition in which there are 3 or 4 long rays and 4 short ones is by far the most common; and further, that the cases in which there are either 3 or 4 long rays are almost equally abundant. It seems not unlikely, then, that *A. tenuispina* usually has 7 rays before it divides, and that it divides into two parts having, respectively, 3 and 4 rays, the division-surface then giving rise, in the greater number of cases, to 4 new rays, but sometimes to 5, 3 or even 2.

If all the individuals observed had undergone autotomy and regeneration according to this scheme, then those with 7 and those with 8 rays would be expected to occur in equal abundance; 8 is next in frequency to 7, but the latter preponderates because some starfishes have probably not autotomized at all, and because all the animals which have divided do not adhere to this paradigm (see Fig. 11). Yet, in the majority of cases, 4 rays are regenerated whether there are 2, 3 or 4 long (old) rays in evidence.

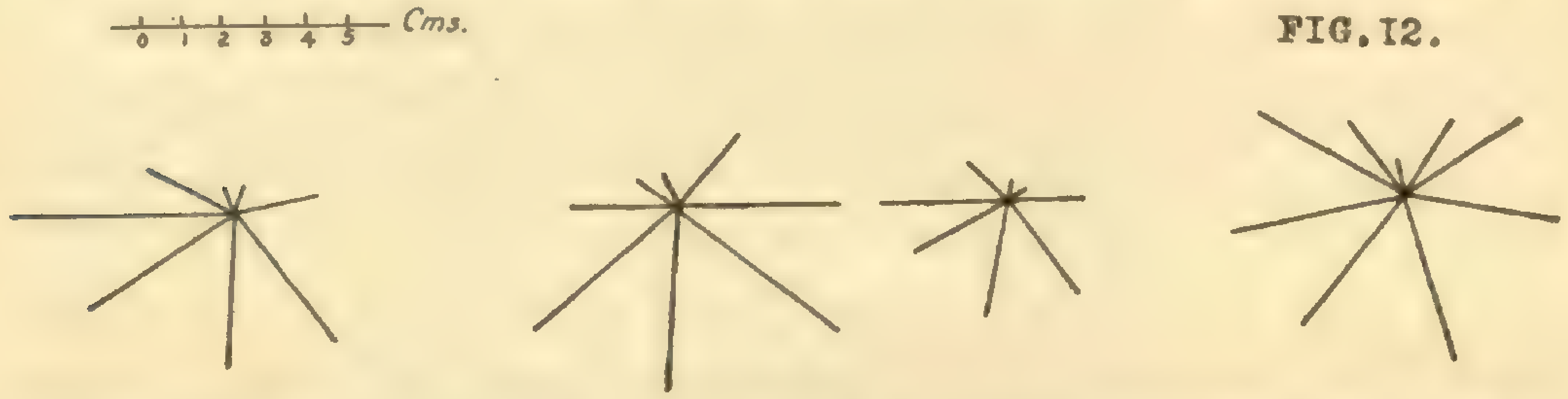
It would seem that self-division may occur at any time in the life history of *A. tenuispina*, or at least in animals of all sizes, though it is my impression, gained from handling many live individuals, that the smaller (younger ?) ones autotomize more readily than larger ones. Those showing two distinct ray groups ranged in longer ray length from 11 mm. to 65 mm. There is no evidence that autotomous divisions follow one another rapidly, or indeed that they occur more than once in any given individual.

One case was observed in which there was one long ray only, and 6 shorter ones. This may mean that a single ray can regenerate the whole body, as suggested by v.

Martens (1866, quoted by King, 1898) for this species. I have not been able to substantiate this idea by laboratory experiments, for, in my tests, single isolated rays did not live more than a few weeks.

(*b*) Newly forming rays have a tendency to appear in symmetrically disposed pairs (see Fig. 12), which gives to

FIG. 12.



Diagramatic Representation of the Rays of Four Asterias, Showing Tendency of Rays to Appear in Pairs. Measured from the mouth along the ventral side.

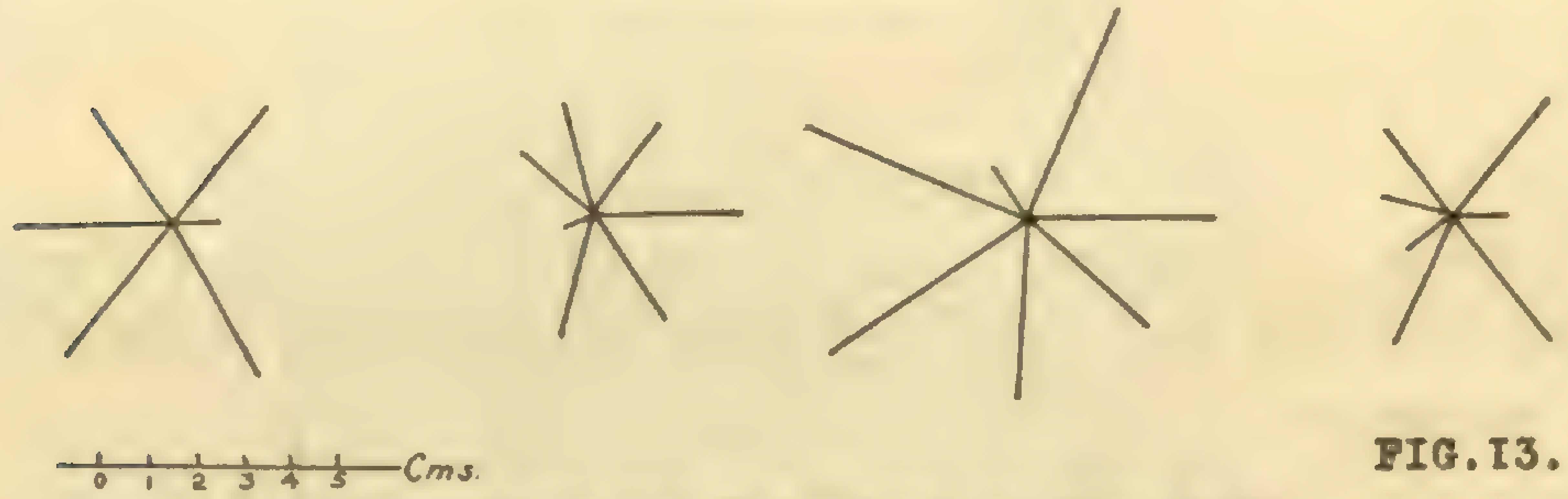


FIG. 13.

Illustrating the Relations of New Rays.

many individuals a strikingly bilateral aspect. This is accentuated by their behavior, for, in the absence of directive stimuli, they commonly move with the longest rays in advance. In moving away from the light, the locomotor movement of the group of longer rays also tends, in many cases, to produce a spurious "orientation." When placed oral side up, the larger rays exert a determining influence on the direction and manner of righting. These effects are due to the greater pedicel and muscle development of the longer, thicker, rays.

The formation of two rays at a radial cut on the disc was found by King (1900) in *Asterias vulgaris*.

V. I have suggested, above, that *Asterias* with 7 subequal rays have probably arrived at that condition by different routes. One method of ray multiplication appears to be the spontaneous addition of new rays at any point on the disc. Twelve starfish were found which showed but one ray markedly shorter than the others.

Of these, 4 had 5 long rays, 4 had 6, 2 had 7, 1 had 3 and 1 had 4 (see Fig. 11). The addition of new rays during adult life is, so far as known, unusual among starfish, excepting in the multiradiate forms (cf. Ritter and Crocker, 1900; Clark, 1907; M'Intosh, 1907). The twelve cases found in *A. tenuispina* may mean merely that a single ray has been cast off and is being regenerated, for there is found about the same percentage of naturally occurring regenerating examples of *A. vulgaris* (King, 1898; 1900). Yet I am inclined to interpret this condition as indicating the way in which the modal hepta-radiate form is derived from the fundamental penta-radiate one, or from a hexa-radiate plan, if the young of *A. tenuispina* be like the post-larvæ of *Pycnopodia* (Ritter and Crocker, 1900) previous to self-division.[3] The three smallest *Asterias* seen had 6 rays. These were subequal and 8 $\pm$ mm. long. Other specimens, slightly larger, had either 7 or 8 rays.

Cases such as those illustrated in Fig. 13 may further prove that addition of new rays occurs independently of the reformation of rays subsequent to self-division.

VI. The number of madreporites in *A. tenuispina* is also variable, as noted by Ludwig (1897, p. 358) and others. The number of madreporic bodies is certainly not correlated with the size of the starfish. One of the smallest ones seen had 8 rays and 5 madreporites, its mean ray length being 10 mm.; while the largest animal collected had 5 rays, with a mean ray length of 70 mm., and but one madreporite. The table in Fig. 14, which includes all cases in which the madreporites were counted, shows that, while the distribution of these bodies is irregular, their number is to some extent correlated with the number of rays. Ludwig gave it as his opinion that there was no correlation of this sort. The relation stands out more clearly if only those individuals having equal rays (and therefore presumably "full grown") are included (Fig. 15). Unfortunately, the number of animals is small.

Multiple madreporites were noted in 5 out of 101 ex-

[3] According to Clark's (1907) studies, the young *Heliaster* has five rays only; his results throw considerable doubt upon the correctness of the conclusions of Ritter and Crocker.

amples. Three of these showed a condition which might have arisen either by the fusion of two plates or by the

NUMBER OF RAYS

NUMBER OF MADREPORITES	2	3	4	5	6	7	8	9	
1	1	1	2	3	5	6	4		22
2		1	1	3	7	10	4	1	27
3					4	10	14		28
4					2	8	10	2	22
5					1		1		2
	1	2	3	6	19	34	33	3	101

FIG. 14

Correlation between number of madreporites and number of rays

NUMBER OF RAYS

NUMBER OF MADREPORITES	4	5	6	7	8	9	
1		2	1	1			4
2	1	2	1	2			6
3			1	4	2		7
4			1		2	1	4
	1	4	4	7	4	1	21

FIG. 15

Relation of ray frequency to number of madreporites in animals with rays of equal length

division of a single one. The other two cases were similar, but of trefoil form. Dissection showed, in each instance, that a single stone canal was present. Therefore these multiple plates had probably arisen by the division of an originally single one. (For a similar condition in *A. vulgaris,* see Davenport [1901].) Only one multiple madreporite was found in any one individual.

Summary

1. The modal number of rays in *Asterias tenuispina* is 7. The range in ray number is from 2 to 9.

2. The 7-ray condition is uniformly the most frequent, even in widely separated localities.

3. The modal ray number is the same for animals with subequal rays as for those with a group of regenerating rays.

4. The evidence indicates that, *most commonly, A.*

tenuispina has 7 rays before it undergoes autotomy, that it divides into 3-ray and 4-ray portions, and that each of these parts regenerates 4 rays.

5. Regenerating rays tend to appear in bilaterally disposed pairs, as regards size.

6. There is no evidence that self-division occurs often in the life of individuals, though possibly it does.

7. New rays may be added at any point on the disc.

8. The number of madreporites varies from 1 to 5, and is to some extent correlated with the number of rays; it is not correlated with the size of the animal.

9. Double or triple madreporites occur in about 5 per cent. of the individuals.

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[1] "Phataria" is an error, as pointed out by Clark (1913).

SHORTER ARTICLES AND DISCUSSION

MR. MULLER ON THE CONSTANCY OF MENDELIAN FACTORS

In discussing the selection experiments of Phillips and myself with hooded rats,[1] Mr. Muller[2] accepts the explanation of "modifying factors" which we offered to account for certain peculiar results obtained, but rejects the idea which we also suggested, that the chief genetic factor concerned may be undergoing quantitative variation. He rejects it on the ground that this explanation is not "in harmony with the results of Johannsen and other investigators." The work of Johannsen with seed-size in beans and the work of others with *Drosophila* is cited in support of this statement.

It is difficult to understand how the experiments of Johannsen have any direct bearing on the case since no single Mendelizing unit-factor was demonstrated in that connection; but in the hooded pattern of rats a Mendelizing unit-factor is unmistakably present and it is the quantitative variation of this which is under discussion, not the presence of many or few additional factors, concerning which Muller adopts our explanation. Appeal to the work of Johannsen with bean-size to show that our conclusions concerning color pattern in rats are incorrect is illogical because the cases are not parallel. The citation by Muller of the work on rabbit-size by MacDowell and myself[3] is equally non-germane, because no demonstrable Mendelizing unit-factor is involved in that case either. He might with propriety cite the bean work as bearing on the interpretation of the inheritance of body size in animals, or *vice versa,* since both involve blending inheritance. But neither of these cases has any direct bearing on the question of unit-character constancy, since in neither case has a unit-character, either constant or inconstant, been shown to exist.

The citation of work with *Drosophila* is more to the point, since the "mutations" of *Drosophila* Mendelize. But is it certain that they do not vary? Muller admits that they do *occasionally* vary, stating that "in one case (possibly in two or three cases)

[1] Castle and Phillips, "Piebald Rats and Selection," Publ. No. 195, Carnegie Institution of Washington.

[2] AMER. NAT., Vol. 48, p. 567.

[3] Publ. No. 196, Carnegie Institution of Washington.

a locus has mutated three times, each time in a different way." He does not think that smaller changes than these have occurred, since "much smaller could easily have been detected." From this statement I infer that the opinion rests on casual inspection rather than measurement, for which reason I do not attach much importance to it. The hooded pattern of rats was not supposed to vary quantitatively until its quantitative study was undertaken. Two types of hooded rats were recognized, one more extensively pigmented than the other, and these were supposed to be discontinuous like the several "mutations of a locus" in *Drosophila*. Quantitative study has completely dispelled this idea as regards the hooded pattern of rats, and I have no doubt the same would be true of *Drosophila*. How easy it is to be sure of a thing which has not yet been investigated, so sure that investigation of it is considered a waste of time. Muller is confident that such variation as occurs in *Drosophila* "can not even remotely be compared to fluctuating variability," and he generalizes thus:

> "In no known case do the variations of a gene among, let us say, several thousand immediate descendants of the individual possessing it, form a probability curve."

The use of the word "gene" in this sweeping statement safeguards the author, since no one, so far as I know, claims ever to have seen a "gene" or to have measured it. How could the "variations of a gene" be expected to "form a probability curve" if the gene is not measurable? But if the author will allow the substitution of *visible character* for "gene" in his challenge, I will gladly accept it and I will add this generalization for his consideration—*No one has by actual observation and measurement shown the existence of any visible character in any animal which is not quantitatively variable.*

As regards the mutations of *Drosophila* which Muller is confident (apparently without having studied the matter himself) do not vary so as to form a probability curve, I had sufficient curiosity some months ago to suggest a quantitative study by one of my pupils, Mr. D. H. Wenrich. Mr. Wenrich studied the wing-length of flies from a culture kindly supplied me by Professor Morgan under the name "vestigial." In advance of a more detailed publication, Mr. Wenrich kindly permits me to state the following facts. The wing length measured in ocular micrometer units was found to vary as follows:

Classes	25–29	30–34	35–39	40–44	45–49
Frequencies	6	34	67	43	13
Classes		50–54	55–59	60–64	65–69
Frequencies		1	1	0	1

The wing-length manifestly varies so as to form a pretty good probability curve; what the "gene" is doing, I do not undertake to say.

It is, of course, conceivable that the variation here observed in actual wing length might be due to variation in general body size, larger flies having longer wings. To determine this point measurements of tibia-length were made on the same flies, and in the case of each individual the ratio was computed between wing-length and tibia-length. These ratios are distributed as follows:

Ratios	.70–.79	.80–.89	.90–.99	1.00–1.09	1.10–1.19	1.20–1.29
Frequencies	2	7	26	49	47	23
Ratios		1.30–1.39	1.40–1.49	1.50–1.59	1.60–1.69	1.70–1.79
Frequencies		7	3	0	0	2

It is evident that there is no constant relation between wing-length and tibia-length, and so between wing-length and general size, with which tibia-length is closely correlated. Again we obtain a good probability curve. Does the "gene" vary or are we dealing also with additional modifying "genes"? We are confronted here with the same problem as in the case of the rats.

But it is possible to assume that the considerable variation shown by vestigial wings in *Drosophila* is purely somatic, "phenotypic," not due to genetic causes, and so would not show any effects if subjected to selection. So it was thought in the case of the plus and minus variations in the hooded pattern of rats, *before the experiment was made,* but experiment has shown, even to Mr. Muller's satisfaction, that the variations are in part due to genetic causes and that selection slowly and surely changes the range of variability. Is it safe to assume the contrary for *Drosophila* in the absence of all experiment?

Mr. Wenrich has also studied the wing-length of "extracted" vestigial flies obtained in the second generation from a cross between pure vestigials and normal flies, and he finds that the variability is regularly increased as compared with that of the uncrossed vestigial race. This again is parallel with what occurs when hooded rats are crossed with wild or with Irish rats, and indicates that similar causes are at work in the two cases. Such

cases present to the genotype theory the following dilemma. Either *one* gene is concerned in the case or many genes. If one only is concerned, it is variable. If many genes are concerned, they are so numerous (whether or not constant) that they present to the observer of the visible character affected a continuous variation series, one capable of indefinite displacement up or down the quantitative scale. The supposed distinction between continuous and discontinuous variation then vanishes. Selection in that case meets with no "*fixed limit*" beyond which it cannot go.

Mr. Muller is seriously disturbed (p. 573) because we are willing to consider it possible that the "factor for hooded" may be contaminated by "its allelomorph (the factor for self)" while associated with it in the zygote represented by the F_1 rats. (The evidence of modification is unmistakable, however one attempts to explain it.) He says this is "violating one of the most fundamental principles of genetics—the non-mixing of factors—in order to support a violation of another fundamental principle—the constancy of factors." Now, when, I should like to inquire, did these principles become "fundamental"; by whom were they established and on what evidence do they rest? I should suppose that Bateson, president of the British Association, might be considered fairly well posted on the "principles of genetics," but neither in his earliest papers nor in his latest do we find any mention of these sacred principles. In his recent presidential address[4] he frankly states his belief that segregation is often imperfect and that "fractionation" of factors frequently occurs as a result of crossing.

We shall look in vain, I think, for those "principles" outside of the "Exakten Erblichkeitslehre" (or its imitations), and when we inquire as to the experimental basis of the principles in question we are met with the satisfied reply, "Johannsen's beans." What a slender basis and what and absurd one from which to derive the "fundamental principle" that Mendelian factors are constant! Yet to date this case, which admittedly involves no clear Mendelian factor, is the only evidence worth mentioning in favor of the constancy *of Mendelian factors!* Do biologists take themselves seriously when they reason thus? Certainly no one else will long take them seriously.

Finally, I may be permitted to correct two misapprehensions

[4] *Science*, August 28, 1914.

into which Muller in common with the Hagedoorns[5] has fallen, viz., (1) that individual pedigrees were not recorded in the course of our selection experiments and (2) that no considerable amount of inbreeding occurred in our work.

It has been our invariable practise, upon recording the birth of an animal and its grade, to record on the same line of the ledger the record number of its mother and father. This enables one in any particular case to trace back the pedigree to the very beginning of our experiments. We have spent much time writing out and studying individual pedigrees, but without discovering any evidence of pure or prepotent lines or individuals, except in a single case, that of our "mutant" series, the origin and complete history of which we have described in detail. The pedigrees, however, of our rats are on record available for study at any time; their full publication would be a quite impossible undertaking.

That extensive and intensive inbreeding has occurred in our experiments will be obvious when I state that all our animals were descended from a very small initial stock, less than a dozen individuals, that from the beginning we have made the most extreme selections possible, mating like with like, never hesitating to mate brother with sister, and putting aside for strict brother-sister matings any litter of young which seemed especially promising. I may say that in no single case (except that of the "mutant" series) have these "special" pens given us advancement obviously greater or less than that of the general selection series of which they formed a part. Nevertheless, we are still continuing to follow them up and will later publish a detailed account of them. Finally I would call attention to pp. 20 and 21 with Tables 48–49 of our full publication, in which are described the hooded offspring of a single selected hooded and a single wild rat. The hooded and the wild rat produced several young resembling the latter, that is, not hooded; these were mated *inter se,* brother with sister. Among the grandchildren (F_2) occurred the usual 25 per cent. of recessives, hooded. Two males were selected from these and mated with females of as nearly the same grade as were available. This process was repeated through seven generations in succession. Seven times animals of like grade were mated together, brother

[5] *Zeit. f. ind. Abst. u. Vererbungslehre,* 11, p. 145. See also my reply in the same journal, 12, p.

with sister when possible, less often brother with half-sister, rarely cousin with cousin. In this way were obtained 804 young from rigidly selected, closely inbred descendants of a single pair of rats, the series extending into generation F_8. We have shown (*l. c.*, p. 21) that the progress of selection within this inbred family follows a remarkably close parallel, generation by generation, to the progress of selection in our plus series as a whole. Muller's anticipation that a different result would follow close inbreeding is not justified by our observations.

In discussing this experiment (p. 21) we have italicized the statement that (so far as the hooded character is concerned) *the entire series is derived from a single hooded individual!* When the Hagedoorns made the statement that our stock had not been sufficiently inbred, they had apparently not seen our full publication and so had no means of knowing to what extent it had been inbred, but Muller, with our full publication before him, apparently repeats the statement without taking the trouble to verify it.

W. E. CASTLE

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October 23, 1914

NO CROSSING OVER IN THE FEMALE OF THE SILKWORM MOTH

IN a recent review[1] of a paper by Y. Tanaka[2] on linkage in the silkworm moth, I pointed out that some of his data suggested that crossing over was occurring in only one sex. While the data were not sufficient to establish this conclusion, there was at this time another paper by the same author[3] which I had not seen. In this paper are presented data which clear up the matter.

Tanaka has now made back-cross tests of both sexes. That crossing over does occur in the males was shown by the mating sysy ♀ × SYsy ♂, which gave a total of 865 cross-overs among 2,907 offspring. The cross sysy ♀ × SysY ♂ gave 151/488 as the proportion of cross-overs. But when females were tested, SYsy ♀ × sysy ♂ gave no cross-overs in 1,183 offspring. Tanaka refers to another paper, apparently in press, in which he has shown the same relations (*i. e.*, crossing over in males, none in

1 AMER. NAT., XLVIII, 1914.

2 *Jour. Coll. Agr. Tohoku Imp. Univ. Sapporo*, V, 1913.

3 *Jour. Coll. Agr. Tohoku Imp. Univ. Sapporo*, VI, 1914.

females) for the combinations NynY and MYmy. As stated in my former review, there was in the earlier paper a record of the mating sysy ♀ × SysY ♂, giving no cross-overs in 128 offspring. Tanaka now says, referring to this case: "Whether there may exist, in certain occasion, a complete reduplication [linkage] in *male*, or whether the above result is due to any mistake by which sex-signs have been reversed, is at present uncertain. No similar case has as yet been found in other families."

The evidence seems to make it highly probable that crossing over in the silkworm moth occurs only in the male; a surprising result when we remember that in *Drosophila* it occurs only in the *female*. One is immediately reminded that in *Drosophila* the male is heterozygous for the sex-differentiator, while in *Abraxas* and probably all moths the female is the heterozygous sex. These facts are highly suggestive, and lead one to wonder what will be found with regard to crossing over in the two sexes in birds and mammals, where similar differences in sex-determination occur. Another point worth noting in this connection is that in the hermaphroditic sweet pea and *Primula* crossing over occurs in the formation both of pollen and of ovules.

Tanaka reports two cases of aberrant results which, as he says, may be explained as due to mutation ("dropping out") of S in one case, and of both S and Y in the other. He adds that such an assumption is premature. To the writer it seems more probable that the females involved were not virgin. The results are easily explained on the assumption that they had paired with brothers before isolation, since brothers of the necessary composition are shown by the pedigrees to have been present in each case.

Another interesting point brought out by Tanaka's more recent paper is the relation between the larval patterns known as striped, moricaud, normal, and plain. In my earlier review I followed Tanaka in treating these patterns as affected by three pairs of genes: S (striped) and s, M (moricaud) and m, and N (normal) and n, plain being the triple recessive. The same scheme has been followed in the early part of this paper. On this assumption, as Tanaka points out, it is necessary to suppose that complete linkage occurs between these three pairs of genes. The evidence need not be gone over in detail here, but there are over 10,000 larvæ recorded from various tests of this relation, without a single cross-over among them. Although Tanaka does not mention the point, this at once brings up the possibility that

we may be dealing with a system of multiple allelomorphs. No two of the types when mated together give a third in F_1; and, unless one or both carry a recessive in heterozygous form, any two types give a 3:1 ratio in F_2, or 1:1 on back-crossing to a recessive. The four patterns involved seem, from the descriptions, to fall roughly into a series in the order striped, moricaud, normal, and plain. That is to say, the second two are rather intermediate in appearance between striped and plain. Although I believe any arguments as to the nature of genes which are based on the appearance of characters are open to very serious objections, it must still be admitted that the different characters involved in a case of multiple allelomorphism are generally of the same sort.[4]

On the chromosome view, if the genes just discussed are allelomorphs they occupy identical loci in homologous chromosomes. If they are not allelomorphic but closely linked, they occupy different but closely opposed loci in homologous chromosomes. In either case, any combination of them should give approximately the same linkage to the Y–y pair of genes, which occupy a locus in the same chromosome, but some distance away. The linkage of the striped-normal, striped-plain, and moricaud-plain combinations with the Y–y locus appear from Tanaka's data to be in fact about the same, though the data on the first (striped-normal) are the only ones sufficiently large to be very significant.

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October, 1914

THE INFLUENCE OF POSITION IN THE POD UPON THE WEIGHT OF THE BEAN SEED

IN a note on the pure line problem Belling[1] has emphasized the significance of position in the pod as a factor in determining the weight of the bean seed. Since this point in his paper seems to have attracted some attention among those interested in genetics, it may not be out of place to call attention to a series of quantitative determinations of the intensity of the relationship[2] and to illustrate the results secured.

If one numbers the successive ovules of the pod from 1 up,

[4] I have discussed this aspect of the matter briefly in another paper (AMER. NAT., XLVII, 1913, p. 237).

[1] Belling, J., "Selection in Pure Lines," *Amer. Breed. Mag.*, 3: 311–312, 1912.

[2] Harris, J. Arthur, "A Quantitative Study of the Factors Influencing the

he may regard the numbers as measures (in units of intervals between adjacent ovules) of the distance of ovules from the

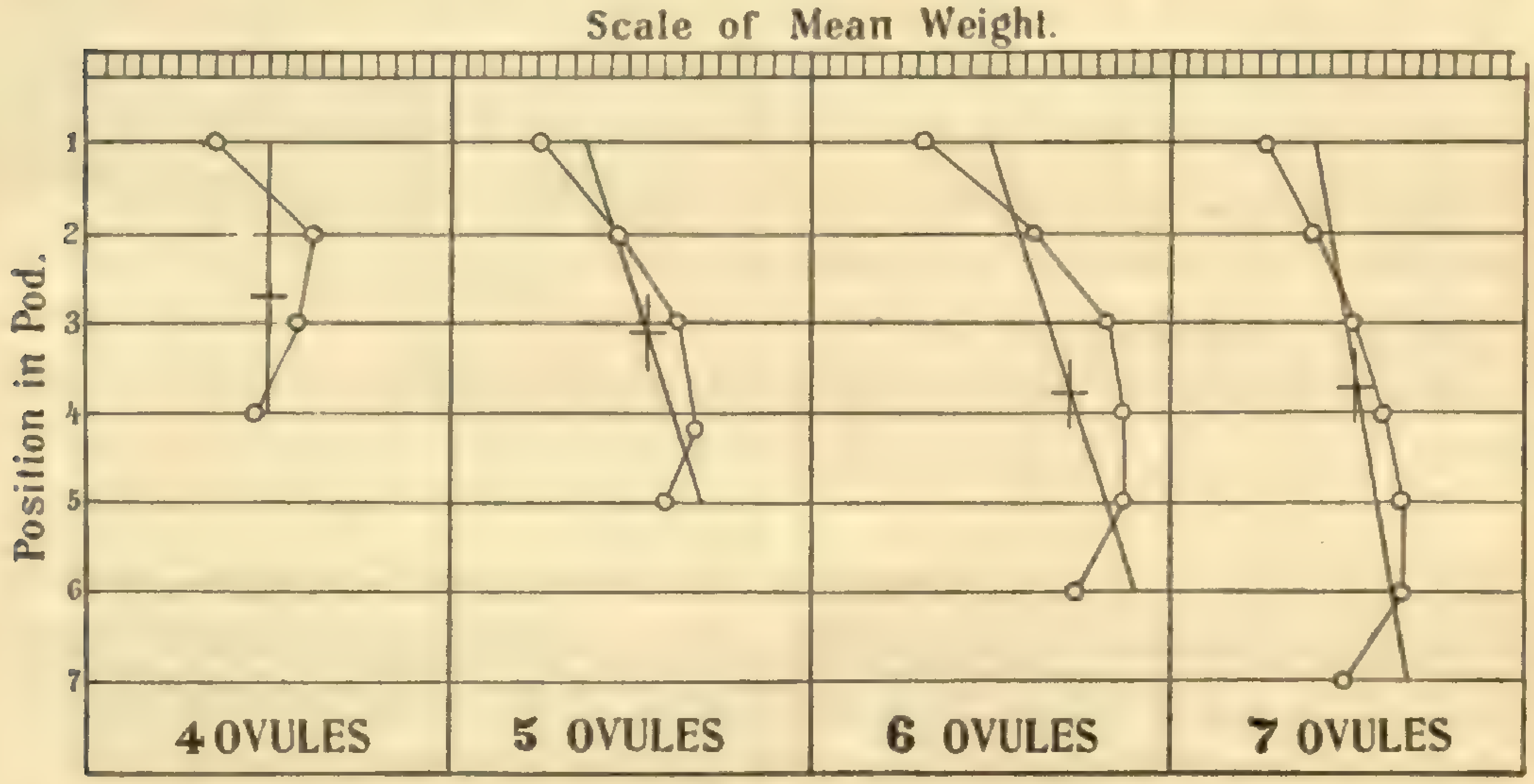

FIG. 1.

proximal end of the pod, and may then express in terms of correlation the relationship between the weight of the seed and its position in the pod.

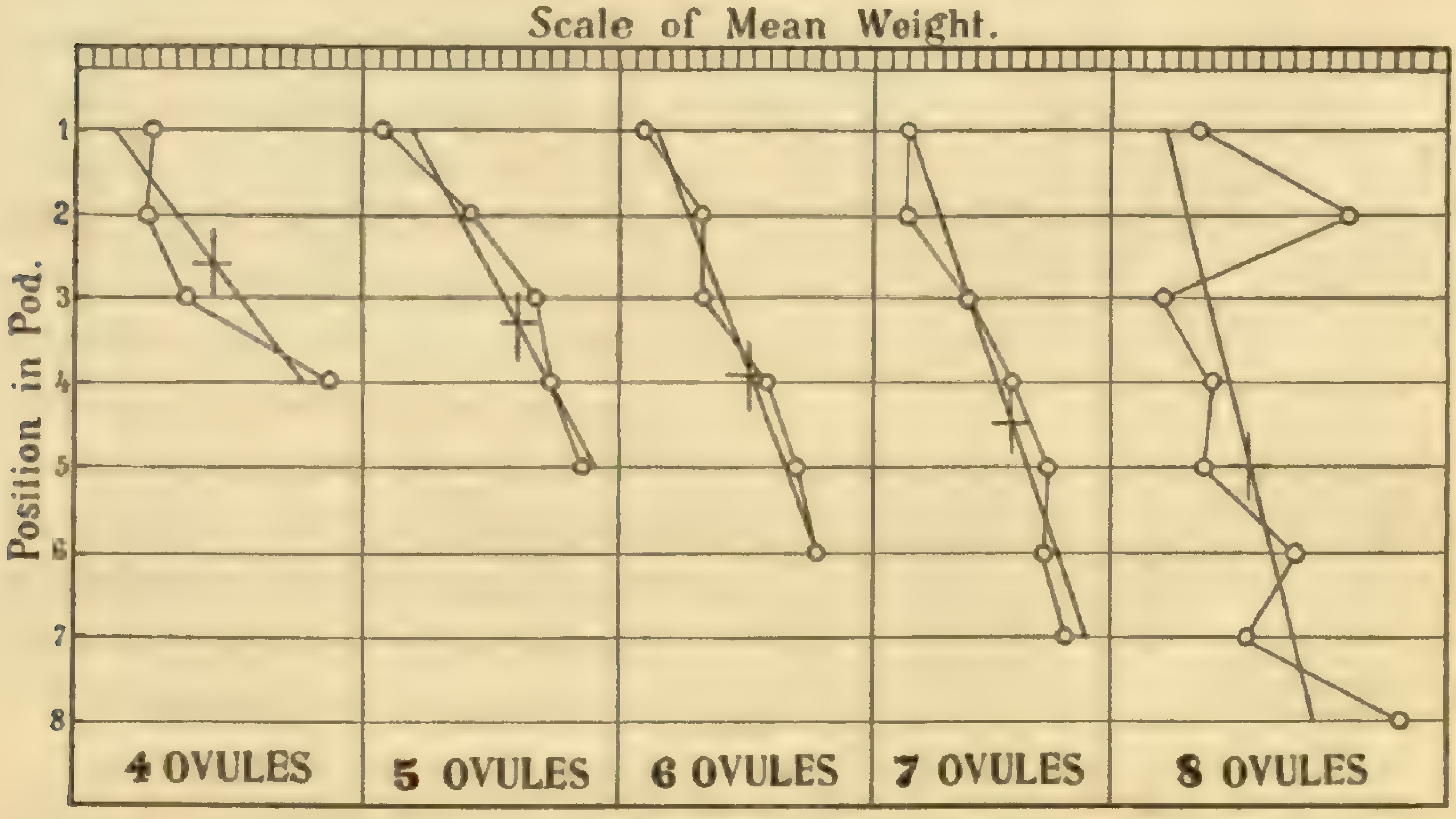

FIG. 2.

In doing this, the pods should of course be sorted into classes according to the number of ovules which they produce and the relationship computed for each group of pods separately, for there is no reason for believing that the fourth in a pod with 4 ovules is comparable with the fourth seed in a pod with six. This

Weight of the Bean Seed. I. Intra-Ovarial Correlations,'' *Beih. Bot. Centralbl. Abt.*, I, 31: 1–12, Pl. 1–4, 1913.

has now been done for twenty series of pods, drawn from five cultures belonging to three distinct varieties (Navy, Golden Wax and Burpee's Stringless) and embracing altogether about 23,000 individually weighed seeds. In every one of these cases a positive correlation has been found, *i.e.*, the weight of the seed increases as its distance from the base of the pod becomes greater. The intensity of this interdependence is, however, not very great, at least in the varieties so far studied. The correlations range from $.014 \pm .046$ to $.238 \pm .068$, with an average value of about .132, or about 13 per cent. of perfect correlation.

The rate of change has been expressed by the slope of a straight line for four different classes of pods studied for a culture of Navy beans made at Sharpsburg, Ohio, in 1907 (Diagram 1[3]) and for five classes from a culture of Burpee's Stringless beans grown at the Missouri Botanical Garden in the same year.

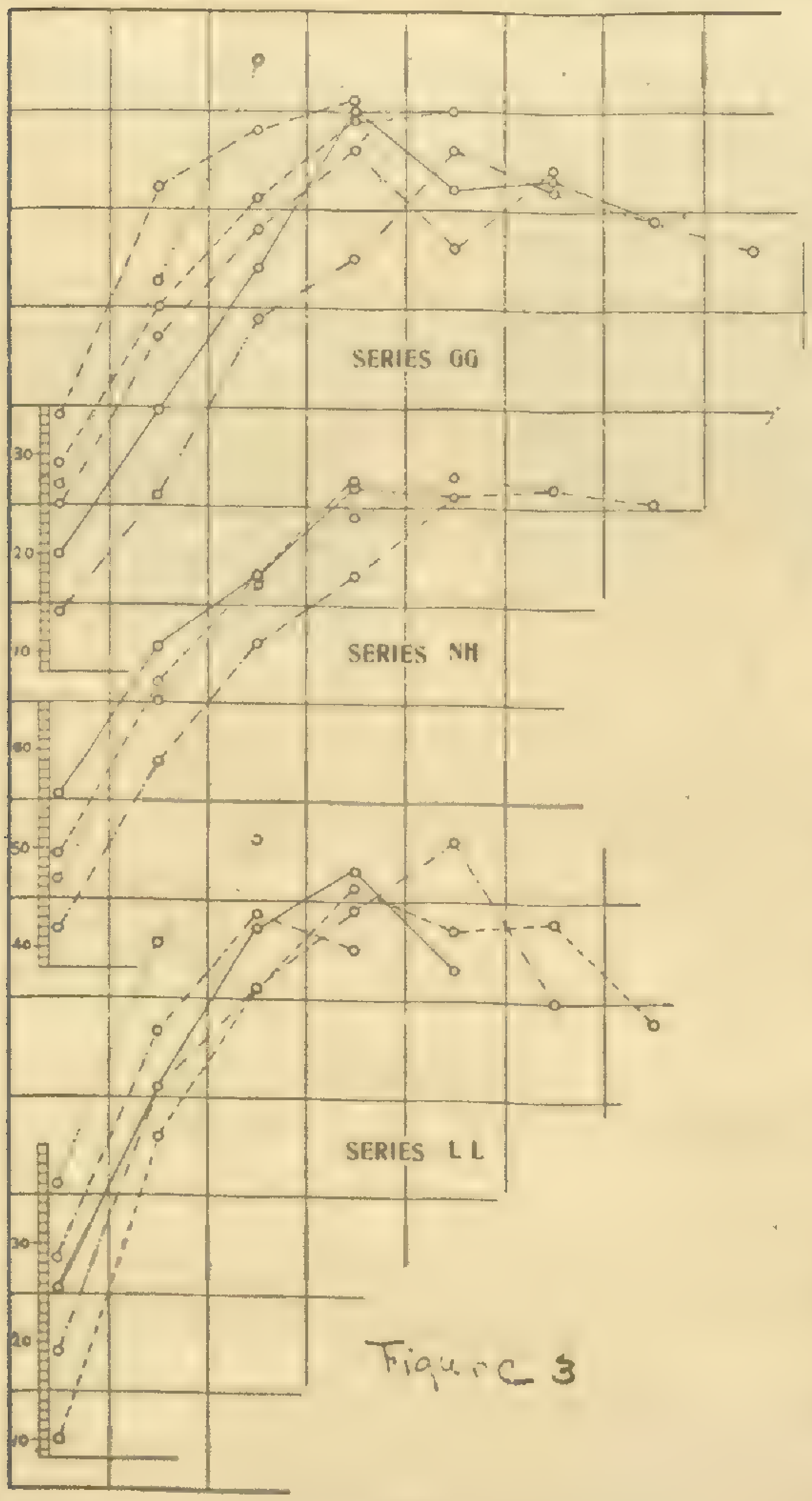

FIG. 3.

In the first of these, the Navy series, it appears that the observed mean weights at first increase rather rapidly, then the rate of increase falls off and finally the seeds nearest the tip (distal or "blossom" end of the pod) become somewhat lighter than those a little lower down. Here a curve would fit the observed means better than a straight line. In the Burpee's Stringless culture (Fig. 2) however, the change in seed weight can for all practical purposes be represented by a straight line as well as by any curve.

The percentage of ovules which develop into seeds also increases from the base toward the stigmatic end of the pod. In small pods the rate of increase may be fairly regular, but in larger pods

[3] In the diagram for this series published in the original paper there is a slip in the representation of the slope of the line for pods with 4 ovules.

it falls off toward the stigmatic end, where the fecundity may be even lower than it is a little farther down in the pod. This is admirably shown in Diagram 3, in which *GO* stands for a series of Burpee's Stringless grown at the Missouri Botanical Garden, *NH* for a series of Navy beans grown at Sharpsburg, Ohio, and *LL* for a series of Golden Wax beans grown at Lawrence, Kansas. All were grown in 1907. Here the percentage of development of ovules at different positions in the pod is shown for the different classes of pods by the scales to the left of the figures. The reader may ascertain the class of pods represented by any particular curve by noting the number of circles representing percentage development in the various positions. These correspond to the number of ovules per pod. In the diagrams the positions (abscissæ) from left to right represent the positions from the base to the tip of the pod.

J. Arthur Harris

ANOTHER GENE IN THE FOURTH CHROMOSOME OF DROSOPHILA

Until the appearance of bent wings, only three groups of linked genes had been found in *Drosophila amelophila,* although four pairs of chromosomes had been identified in the diploid group. Since the character bent wings, worked out by Mr. H. J. Muller, was found to be unassociated with any of the three groups, the gene producing this character was said to be located in the fourth chromosome.

Recently a new character, designated as eyeless, appeared. Flies having this character either lacked eye pigment and ommatidia or had one or both eyes reduced in size. All of the pure stock showed some loss of eye structures. Eyeless is recessive to the normal eye. In order to determine the linkage, eyeless males were crossed in turn to females of the stocks at Columbia University. These stocks representing the three groups were (1) miniature wings, (2) black body and vestigial wings, and (3) spread wings. The genes producing these characters are in the first, second and third chromosomes, respectively. The F_1 from all three crosses had normal eyes. They were inbred in each case and gave the following.

The equation should be $w = 9.987 + .021\ p$. The line as it appears here is correctly drawn.

Cross 1. Miniature ♀ by Eyeless ♂

	Normal Long	Normal Miniature	Eyeless Long	Eyeless Miniature
F_2.	1142	1106	245	193

Since the eyeless flies were females as well as males, the character eyeless is shown not to be a sex-linked character; for, if it were, it would be inherited only by the grandsons of the eyeless male. Since the eyeless flies are not nearly as viable as the wild stock, the eyeless classes fall below the expectation.

Cross 2. Black Vestigial ♀ by Eyeless ♂

	Normal Long	Normal Vestigial	Eyeless Long	Eyeless Vestigial
F_2.	1303	417	278	97

The same count, when grouped according to the body color, was as follows:

	Normal Gray	Normal Black	Eyeless Grey	Eyeless Black
F_2.	1289	431	293	82

Cross 3. Spread ♀ by Eyeless ♂

	Normal not Spread	Normal Spread	Eyeless not Spread	Eyeless Spread
F_2.	1349	373	300	76

Allowing for the decreased viability of eyeless, both of the preceding crosses may be regarded as 9:3:3:1 ratios. Hence they show that there is no linkage of eyeless with the characters whose genes are in the second and third chromosomes.

Eyeless females were then crossed to bent-winged males (Cross 4). No bent eyeless flies were produced in the F_2. As the count was small, the F_2 bent flies were crossed to the F_2 eyeless, and then the F_3 normal, which had the same germinal constitution as the F_1, were inbred to give F_4, which should give the same results as the F_2.

Cross 4. Bent ♂ by Eyeless ♀

	Normal not Bent	Normal Bent	Eyeless not Bent	Eyeless Bent
F_2.	596	193	195	0
F_4.	741	172	131	0
Total	1337	365	326	0

Since an approximate 2:1:1:0 ratio, instead of a 9:3:3:1 ratio, was realized, the conclusion that eyeless and bent belong

to the same group and in this sense may be said to be in the same chromosome pair is evident. Until a bent eyeless fly—a cross over—is obtained, the amount of crossing over between these two characters in the fourth chromosome can not be directly determined.

MILDRED A. HOGE

AN ABNORMAL HEN'S EGG

In a frequently quoted paper, Parker ('06) has classified double eggs on the basis of the factors supposedly concerned in their formation. Considering the ovarian and oviducal factors as independent, Parker says:

> As a result of these two factors, three classes of double eggs can be distinguished; first, those whose yolks have come from an abnormal ovary but have passed through a normal oviduct; secondly, those whose yolks have come from a normal ovary but have passed through an abnormal oviduct; and finally those produced by an ovary and oviduct both of which have been abnormal in their action.

FIG. 1. PHOTOGRAPH OF THE SPECIMEN × 1.

Cases of ovum in ovo have been attributed by Parker and others to antiperistalsis. Patterson ('11) mentions a case of an inclosed double egg in which there were two distinct peristaltic actions. Féré ('98) has called attention to the fact that hens frequently lay several double eggs in succession. Féré claims that he succeeded in producing double eggs in a hen which normally laid single eggs, by drugging her with atropine sulphate. Glaser ('13) has described the ovary of a hen which habitually laid double eggs and concludes that fusion of the follicles is the explanation of some double eggs.

The case which I wish to record is very similar to that figured by Hargitt ('12) and termed by him a "gourd-shaped" egg. Unfortunately, the egg which Professor Hargitt studied was not preserved carefully and on account of evaporation, the condition was such that he could not be certain of the presence of yolk in the smaller end. He assumed that the egg was comprised of

FIG. 2. DIAGRAMMATIC MESIAL VIEW OF THE ABNORMAL EGG, SHOWING THE RELATION OF THE YOLK TO THE ALBUMEN.

about normal parts in the larger end, and that the smaller consisted of only albumen, "its yellowish tint having resulted from the evaporating process which had taken place."

The egg shown (Fig. 1) was presented to Professor Julius Nelson, of Rutgers College, several years ago and was carefully preserved in a jar of alcohol. The result was that although the action of the alcohol had partially decolorized the yolk, it was possible to trace it throughout the entire extent with no difficulty. As can be readily seen from the photograph, that part of the egg which might be termed the "neck" presented a much roughened appearance from the excessive accretion of lime. A nodule of lime at the smaller end of the shell would seem to indicate that the last deposit of the shell glands was there received.

For convenience in examination of the irregular shaped egg, it was separated at the circling line seen in Fig. 1, and then the two parts were halved with a sharp scalpel, after the penetration of the shell by means of scissors.

When the first separation was made at the line indicated, one could readily discern the presence of a *constricted* yolk surrounded by apparently normal albumen. Examination of the halved portions showed that the yolk extended from the larger end through the constricted region to occupy a position approximately normal in the smaller end. It seems possible that this particular abnormality may have been caused by a constricted

oviduct rather than from the fusion of two eggs during apposition, induced by anti-peristalsis.[1]

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[1] Since the above was written, but before the proof came to hand, an authoritative paper has been published (Maynie R. Curtis, Studies on the Physiology of Reproduction in the Domestic Fowl, Vi. Double- and Triple-Yolked Eggs. Biol. Bull. Vol. 26, pp. 55–83.) in which no mention has been made of the possibility of incomplete separation of both yolk and albumen of a single egg. Evidence of such separation is not wanting in other vertebrates, however scant it may be in the fowl.

SCIENTIFIC LITERATURE

GENETIC DEFINITIONS IN THE NEW STANDARD DICTIONARY

The widely advertised aim of the Funk & Wagnalls Company to include in their "New Standard Dictionary of the English Language" all of the new additions to scientific terminology naturally invites the specialist in each branch of science to examine the definitions of the new words in his own field. Professor Miller[1] has called attention to the fact that the mathematical definitions are not reliable. The same criticism must be made regarding the definitions of many terms now familiar in the literature of genetics. For some of the errors in these definitions the editorial staff can not be blamed, because the errors were passing current among genetic writers themselves, at a time when further changes in the dictionary probably became impossible; other errors are less easily explained. While such a monumental work as the Standard Dictionary tends to fix the usage of language, the shortcomings of the genetic definitions may not be expected to seriously affect the terminology actually used by the specialists in this field; but for those who are engaged in other scientific fields, who have only a casual interest in genetics, and who must, therefore, depend upon the dictionary for the meaning of any genetic terms they may happen to meet, the erroneous definitions are unfortunate. While very few of the genetic definitions are free from defects, either of omission or of commission, only those which seem most obviously defective will be considered here. In the following list of words the definition of the New Standard Dictionary is stated first, and then follows, in italic type, a definition which I believe will meet with the approval of most geneticists.

Acquired. Transmitted by inheritance to subsequent generations; as, *acquired* characters.

Acquired character. *A modification of bodily structure or habit which is impressed on the organism in the course of individual life.*

Both of these definitions occur in the New Standard Dictionary, the first under "acquired," the second under "character." Although "impressed on" may not be the best figure of speech to use in this connection, the second definition represents fairly well the correct usage of this phrase. It is difficult to understand why

[1] *Science*, N. S., 38: 772, November 28, 1913.

essentially the same definition should not have been given at both places.

Allelomorph. "In Mendelian inheritance a pair of contrasted characters which become segregated in the formation of reproductive cells."

Allelomorph. *One of a pair of contrasted characters which are alternative to each other in Mendelian inheritance. Often used with doubtful propriety as a synonym for gene, factor or determiner.*

The defects in the dictionary definition in this case are two: (*a*) The definition is plural, while "allelomorph" is singular; the "allelomorph" is not a *pair* of characters, but a *single* character. (*b*) No segregation of allelomorphs takes place in the formation of asexual reproductive cells.

Allelomorphism. "The presence of allelomorphic pairs of characters."

Allelomorphism. *A relation between two characters, such that the determiners of both do not enter the same gamete, but are separated into sister gametes.*

Alternative inheritance. "The transmission to alternating generations of descendants of the characteristics of either parent, as that of the father to the odd, and of the mother to the even generations."

Alternative inheritance. *A distribution of contrasting parental or ancestral characters among offspring or descendants, such that the individuals exhibit one or other of the characters in question, combinations or blends of these characters being absent or exceptional.*

Biotype. "In Mendelian inheritance a race or strain that breeds true or almost true; a term introduced by Johannsen."

Biotype. *A group of individuals all of which have the same genotype.*

The word "biotype" was introduced into English by Dr. Johannsen[2] in 1906 with the definition "one single 'sort' of organisms." It is a term of general applicability and not limited to Mendelian races, as stated in the New Standard Dictionary. Although homozygous biotypes generally do breed true, this is not an essential feature and therefore should not be included in the definition. Ever-sporting varieties are now well known which do not breed true, but which, so far as present evidence goes, do constitute single homozygous biotypes. Heterozygous biotypes generally do not breed true.

Clon. "A plant-group the members of which have been grown from an original stock, but which do not come true from seed."

Clone. *A group of individuals produced from a single original individual by some process of asexual reproduction, such as division, budding, slipping, grafting, parthenogenesis (when unaccompanied by a reduction of the chromosomes), etc.*

There are several defects in the dictionary definition of this word, even if restricted to a plant-group in accord with the original meaning given to it by Webber, who introduced the word.

[2] Report of the third International Conference of Genetics, p. 98.

The defects consist, first, in the ambiguity of the word "stock," because we may grow plants "from an original stock" of seeds, quite as well as from cuttings, while a clone is derived from a single individual; second, the statement that clones do not come true from seed is incorrect, for a clone formed by cuttings, etc., from a homozygous individual does "breed true," *i. e.*, it produces seedling offspring of its own type. The word is now being generally applied to animals as well as to plants.

Coupling. ("Genetic coupling" is not defined in the dictionary.) *Such a relation between the genes of two unit-characters that they have a more or less marked tendency to be included in the same gamete when the individual is heterozygous for both of the genes in question.*

Cross-over. (Not given a genetic definition in the dictionary.) *A separation into different gametes, of determiners that are usually coupled, and the association of determiners in the same gamete, which are generally allelomorphic.*

Cryptomere. "A plant character which may exist in the germ-cells without making its presence visible."

Cryptomere. *A factor or gene whose presence can not be inferred from an inspection of the individual, but whose existence can be demonstrated by means of suitable crosses.*

The chief defect in the dictionary definition is the restriction of this term to *plant* characters. "Cryptomere" is a general genetic term which may be applied as well to animals as to plants.

Determiner. "The same as determinant 3."

Determiner. *An element or condition in a germ-cell which is essential to the development of a particular feature, quality or manner of reaction of the organism which arises from that germ-cell; a gene or factor.*

The word "determiner," as used in recent years, is not the equivalent of "determinant 3," which latter is correctly defined in the dictionary in terms of Weismann's complicated hypothesis. "Determiner," "factor" and "gene" are now quite generally used interchangeably without implication as to their fundamental nature, simply in the generic sense, as "that which determines."

Dominance. "In the cross-bred offspring of parents with marked mutually antagonistic characteristics, the exhibition by such offspring or its descendants of one of these characteristics to the exclusion of the other."

Dominance. *In Mendelian hybrids the capacity of a character which is derived from only one of the two generating gametes to develop to an extent nearly or quite equal to that exhibited by an individual which has derived the same character from both of the generating gametes. In the absence of dominance the given character of the hybrid usually presents a "blend" or intermediate conldition between the two parents, but may present new features not found in either parent.*

There are several defects in the dictionary definition. In the first place, the parents used in a given cross may not themselves

be homozygous, in which case some of their offspring will resemble one parent and some the other; in such a case, according to the dictionary, both of the contrasted characters would exhibit dominance. The phrase "or its descendants" would make it possible, *in any case,* to include both recessives and dominants, since among the *descendants* of such cross-bred individuals there will also be *recessive* individuals which "exhibit one of the characteristics to the exclusion of the other."

Dominant. "(1) A marked parental character exhibited by a cross-bred organism and its descendants. (2) The parent, cross-bred organism, or descendant exhibiting such character. Parental characters latent in a cross-bred organism, but actively evidenced by its descendants, are called *recessives,* as are the descendants which exhibit them."

Dominant. *(1) A character which exhibits dominance, i. e., that one of two contrasted parental characters which appears in the individuals of the first hybrid generation to the exclusion of the alternative, "recessive," character. (2) An individual possessing a dominant character, in contrast to those individuals which lack that character, which are called "recessives."*

An "*extracted* dominant," as defined in the dictionary, is not distinguishable from the pure homozygous dominant used in the cross from which the dominant in question was "extracted," as no mention is made of the essential historical fact that it is of hybrid origin and that its parent or other known ancestor did not breed true to the same dominant character.

Factors. "Latent physiological units which upon crossing give rise to the new characters found in the hybrid."

Factor. *An independently inheritable element of the genotype whose presence makes possible any specific reaction or the development of any particular unit-character of the organism which possesses that genotype; a gene or determiner.*

The limitation of the term "factor" to those cases in which new characters appear in hybrids, is not in accord with present usage. All the various characters of organisms are to an important degree dependent upon the existence of genotypic factors, regardless of the behavior of these organisms in crosses.

Gen. "A minute hypothetical particle supposed to be the bearer of hereditary qualities."

Gene. *An element of the genotype; a genetic factor; a determiner.*

The treatment of this word in the dictionary is particularly mischievous. When I introduced the word "gene" to English-reading students, I said:[3] "This word is proposed by Dr. Johannsen . . . to denote an internal something or condition upon whose presence an elementary morphological or physiological characteristic depends. The word 'gene' has the advan-

[3] Am. Nat., 43, p. 414, 1909.

tage that it does not assume by its form or derivation any hypothesis as to the ultimate character, origin or behavior of the determining factor.'' In adopting the word ''Gen'' in the German, Johannsen said:[4] ''Das Wort *Gen* ist völlig frei von jeder Hypothese; es drückt nur die sichergestellte Tatsache aus, dass jedenfalls viele Eigenschaften des Organismus durch in den Gameten vorkommende besondere, trennbare und somit selbständige 'Zustände,' 'Grundlagen,' 'Anlagen'—kurz, was wir eben *Gene* nennen wollen—bedingt sind. . . . die Gene sehr vieler Eigenschaften glatt trennbar sind, während andere nicht oder nicht glatt sich trennen. Dies alles erinnert an das Verhalten chemischer Körper. Damit ist aber noch gar nicht gesagt, dass die Gene selbst chemische Gebilde oder Zustände seien—darüber wissen wir vorläufig noch gar nichts.'' How different is all this from ''a minute hypothetical particle''! It is obviously improper, therefore, to define a gene as a ''minute particle.'' Neither is it correct to say that it is ''supposed to be the bearer of hereditary qualities.'' It is only the something of unascertained nature, which must lie at the foundation of any elementary hereditary quality. The spelling ''gene'' is not even mentioned in the dictionary as a *variant*, yet this was the original spelling and is now in practically universal use among geneticists, while no one uses ''gen.''

Genotype. ''A race of organisms different from another in its hereditary qualities; contrasted with *phenotype.*''

Genotype. *The fundamental hereditary constitution or sum of all the genes of an organism.*

The unfortunate definition of ''genotype'' given in the dictionary was current in America at the time when the dictionary forms were probably closed, so that the editors are not in any way to blame for the totally erroneous definition. The definition given by the dictionary for ''genotype'' fits fairly well the word ''biotype.''

Heredity. ''The tendency manifested by an organism to develop in the likeness of a progenitor.''

Heredity. *The distribution of genotypic elements of ancestors among the descendants; the resemblance of an organism to its parents and other ancestors with respect to genotypic constitution.*

The results of modern experimental work on heredity show that the definition given by the dictionary is entirely too restricted. Heredity must be so defined that it may apply to characters that were never exhibited by any ancestor.

Heterozygosity. ''In Mendelian inheritance, the state or condition due to an organism having developed from a heterozygote.''

[4] ''Elemente der exakten Erblichkeitslehre,'' 1. Aufl., 1909, pp. 124–125.

Heterozygosity. *The condition of an organism due to the fact that it is a heterozygote; the state of being heterozygous; the extent to which an individual is heterozygous.*

Heterozygote. "A Mendelian hybrid resulting from the fusion of two gametes that bear different allelomorphs of the same character and which in consequence does not breed true; contrasted with *homozygote.*"

Heterozygote. *A zygotic individual in which any given genetic factor has been derived from only one of the two generating gametes. Both eggs and sperms produced by such an individual are typically of two kinds, half of them containing the gene in question, the rest lacking this gene; consequently the offspring of heterozygotes usually consist of a mixture of individuals, some of which possess the corresponding character while others lack it.*

Homozygosis. "Development from a zygote originating from a union of two gametes of the same kind."

Homozygosis. *The state of being homozygous; the extent to which an individual is homozygous.*

Homozygote. "A zygote formed by the conjugation of two gametes of the same stock; any animal or plant that receives and retains the dominant or recessive characters of both its parents, and is therefore said to be true to type, and breeds true to type."

Homozygote. *An individual in which any given genetic factor is doubly present, due usually to the fact that the two gametes which gave rise to this individual were alike with respect to the determiner, in question. Such an individual, having been formed by the union of like gametes, in turn generally produces gametes of only one kind with respect to the given character, thus giving rise to offspring which are, in this regard, like the parents; in other words, homozygotes usually "breed true." A "positive" homozygote with respect to any character contains a pair of determiners for that character, while a "negative" homozygote lacks this pair of determiners.*

"Two gametes of the same stock" is ambiguous because of the indefiniteness of the word "stock." Many homozygotes receive some dominant *and* some recessive characteristics of the two parents; and what can be intended by the statement that a plant or animal which receives certain characteristics also *"retains"* them? How could it do otherwise?

Hypostasis. (Not given a genetic definition in the dictionary.) *That relation of a gene in which its usual reaction fails to appear because of the masking or inhibitory effect of another gene; contrasted with "epistasis."*

The corresponding adjective "hypostatic" is also not given a genetic definition in the dictionary.

Mendelize. "To cause to follow Mendel's law of inheritance."
Mendelize. *To follow Mendel's law of inheritance.*

The word is rightly indicated in the dictionary, as an intransitive verb; it is manifestly incorrect to define it by the use of a transitive verb.

Mutant. "That which admits of or undergoes mutation or change; specifically, an individual or a species which shows significant changes in form or character in a single generation."

Mutant. *An individual possessing a genotypic character differing from that of its parent or those of its parents, and not derived from them by a normal process of segregation.*

The expression "significant changes" is ambiguous, since every change is significant of something.

Mutate. "To 'sport.'"

Mutate. *To undergo a change in genotypic character independently of normal segregation.*

The word "sport" which is used in the dictionary definition of "mutate" is defined thus: "To vary suddenly or spontaneously from the normal type; said of an animal or plant or of one of its parts." It is well known that many such sudden and spontaneous variations from the normal type are not due to mutations. The word "mutation" is defined in the dictionary as "a permanent transmissible variation in organisms, as distinct from *fluctuation*." This definition is good as far as it goes, but should expressly exclude transmissible variations which are due to normal segregation and recombination of determiners.

Phenotype. "A type or strain of organisms distinguishable from others by some character or characters, whether their observable differences from other organisms be due to their inherent hereditary differences or to the direct action of the environment upon them: contrasted with *genotype*."

Phenotype. *The apparent type of an individual or group of individuals,* i. e., *the sum of the externally obvious characteristics which an individual possesses, or which a group of individuals possesses in common; contrasted with genotype.*

"Phenotype" and "genotype" are both abstractions; the qualities which distinguish the phenotype are always capable of direct observation, while those of the genotype can only be inferred from the results of genetic experiments.

Presence and absence hypothesis, "in the Mendelian doctrine of inheritance, the theory that an allelomorphic pair of characters in every zygote has two contrasted factors or determinants, one representing the positive character of the generated organism and the other denoting its absence."

Presence and absence hypothesis. *The hypothesis that any simple Mendelian difference between two individuals, results solely from the presence of a factor in the genotype of the one individual, which is absent from that of the other. Presence and absence of unit-differences as a convenient* method *of describing the results of genetic experiments should be carefully distinguished from the presence and absence* hypothesis. *The* method *is purely objective and entirely free from hypothetical implications.*

It will be noted that the dictionary definition of this phrase is directly opposite in significance to the one here set forth.

Pure line (Not included in the dictionary.) *A group of individuals derived solely by one or more self-fertilizations from a common homozygous ancestor. Sometimes erroneously applied to groups of individuals believed to be genotypically homogeneous (a homozygous biotype or a clone) without regard to their method of reproduction.*

Repulsion. (Not given a genetic definition in the dictionary.) *Such a relation between two genetic factors that both are not, as a rule, included in the same gamete, referring especially to cases in which the factors in question give rise to obviously different characteristics; also called "spurious allelomorphism."*

Sex-limited inheritance. (Not defined in the dictionary.) *The association of the determiner for any unit-character, with a sex-determiner, in such a manner that the two determiners are either generally included in the same gamete, or that they are generally included in different gametes. This method of inheritance is also called "sex-linked" inheritance by Professor T. H. Morgan and his students.*

Segregate. "To become separated from the rest; specif., of Mendelian hybrids, to separate, by a numerical law, into dominants, hybrids and recessives."

Segregate. *With reference to Mendelian unit-characters, to become separated through the independent distribution of the genetic factors before or at the time of the formation of the gametes.*

The dictionary definition goes too far; the formation of dominants, hybrids and recessives depends not alone upon the fact that the factors *segregate*, but that the segregated factors *recombine*. The word "segregation" receives a fairly satisfactory definition.

Unit-character. (Not included in the dictionary.) *In Mendelian inheritance a character or alternative difference of any kind, which is either present or absent, as a whole, in each individual, and which is capable of becoming associated in new combinations with other unit-characters.*

I have made no systematic study of the definitions of technical terms in other related fields, but have noted incidentally that there is no recognition in the New Standard Dictionary of the generally familiar usage of the words "meristic" and "substantive" as applied to types of variation.

G. H. SHULL

PROFESSOR DE VRIES ON THE PROBABLE ORIGIN OF ŒNOTHERA LAMARCKIANA

IN a recent paper Professor Hugo De Vries[1] has given us the results of a second examination in 1913 of material from the herbarium of Lamarck, and of other sheets of *Œnothera* in the collections of the Muséum d'Histoire Naturelle in Paris. This

[1] De Vries, Hugo, "The Probable Origin of *Œnothera Lamarckiana* Ser., *Bot. Gaz.*, Vol. LVII, p. 345, 1914.

is a very important contribution for there have been some changes in the mounting of important specimens in the herbarium of Lamarck since the first studies by De Vries in 1895 and it was not clear what material formed the subject of his discussion in "Die Mutationstheorie." As the result of this second examination (1913) there can be no misunderstanding of De Vries's conclusion as to what represents the type of *Œnothera Lamarckiana* Seringe, and we have also very positive opinions on the identity of other interesting material in the collections at Paris. Thanks to his descriptions and photographs of these sheets further confusion will be impossible and botanists may now make for themselves the observations that will in the end determine their judgment of the soundness of Professor De Vries's views and of the value of the exceptions that may be taken to them.

I shall not at this time discuss in detail the queries which presented themselves on my reading of De Vries's paper. The most important of the points probably rest on facts that should be shown by the material, but which have not been published in the account of De Vries. I expected to have the data in question this autumn but the European disturbances have necessarily upset my plans and it may be very many months before I can take up the matter.

However, I will briefly say that De Vries's identification of the sheets under consideration are to me not convincing chiefly for the following reasons. His account gives no description of the pubescence of the sepals, stems, or capsules when present. Yet pubescence is a character of great importance in the description of many species of *Œnothera*. To illustrate the point, all races of *O. grandiflora* Solander that I know have sepals and capsules almost glabrous or very sparsely pilose and puberulent. *Lamarckiana* on the contrary presents sepals and capsules with a very heavy puberulent and pilose pubescence. Should any of the specimens at Paris which De Vries has identified with the *Lamarckiana* of his cultures present sepals or capsules lacking the heavy pubescence of this plant the fact to me would be very strong evidence that his identification was incorrect.

There are two sheets under consideration as standing for the type of *Œnothera Lamarckiana* Seringe. De Vries regards one as unequivocally representing the type specimen. I have for various reasons placed the greater emphasis upon the other. Both specimens as shown in photographs appear to have essentially the same features as to their general morphology. Miss

Eastwood and M. Gagnepain who compared the two specimens reported to me that they were very similar. Both were undoubtedly known to Lamarck since the two sheets bear his handwriting, and it is quite possible that Lamarck based his description on both specimens.

The general morphology of these specimens presents several features that are not those of the *Lamarckiana* of De Vries's cultures. Chief among these are (1) the approximate branches, (2) the foliage of narrower and more distinctly petioled leaves, (3) the inflorescence more open and with narrower bracts, (4) the buds more slender and tapering, and apparently with more attenuated sepal tips, (5) the long delicate hypanthium. In these features the specimens are closer to *O. grandiflora* than to *Lamarckiana*. Such morphological characters, it is true, might vary somewhat under different conditions of growth and with the time of collection whether early or late in the season. The pubescence should give us the stronger evidence of relationship since pubescence would be little if at all affected by growth conditions or by season. Of the pubescence on one of these specimens I have Gagnepain's statement that it is close to that of *grandiflora,* but it is only fair to say that no *Œnothera* specialist has reported upon such a comparison as is desired.

Lamarck's description of the capsules of his plant as short and glabrous is a point of great importance. The capsules of De Vries's *Lamarckiana* are certainly not glabrous but they are short. In my contention that Lamarck's plant was a form of *O. grandiflora* Solander I was at first forced to assume that Lamarck must have described immature or partially pollinated capsules. I have, however, this summer grown œnotheras from Mississippi which have the rosettes, habit, foliage, inflorescence, and flowers of *grandiflora,* but which developed glabrous short capsules essentially of the same relative proportions as those of *Lamarckiana*. It is immaterial what is the origin or genetic history of these plants; systematically speaking they represent short-capsuled forms of *O. grandiflora*. Thus we now know of *grandiflora*-like types which even as to their capsules agree with the description of Lamarck. De Vries does not seem to be disturbed by the fact that the material of his cultures presents capsules with a heavy puberulent and pilose pubescence while Lamarck's description specifies a capsule "glabre."

In summary I must say that my opinion remains unchanged with respect to the affinities of the plant described by Lamarck,

Œnothera Lamarckiana Seringe. Whichever of the two specimens considered above represents the type, or if both were concerned in the description, the evidence is to me very strong that Lamarck dealt with forms of *O. grandiflora* Solander. I can see no proof or even reasonable evidence that the *Lamarckiana* of De Vries's cultures agrees with either of the specimens from Lamarck's herbarium. A final judgment, however, should not be made until we have before us details respecting the pubescence of the specimens known to Lamarck.

De Vries is very positive that two other sheets in the collections at Paris present specimens agreeing with his *Lamarckiana*. The first of these (De Vries, Plate XVIII) is from the herbarium of Abbé Pourret and shows material which seems to me to offer very much the same difficulties to an identification with De Vries's *Lamarckiana* as do the specimens of Lamarck. The foliage of lanceolate leaves clearly petioled, the slender tapering buds, the long delicate hypanthium; these are not characters representative of the plants from the cultures of De Vries. They are characters of *O. grandiflora* Solander and should the pubescence prove to be similar to this species I should not hesitate to place these specimens of Abbé Pourret among the forms of *grandiflora*. Until we know the facts of the pubescence, further discussion is unwise, but it does not seem to me that De Vries's identification rests on good evidence.

The remaining sheet at Paris which De Vries (Plate XIX) identifies with his *Lamarckiana* is a plant from the herbarium of André Michaux. De Vries on historic grounds naturally attaches importance to this sheet for if it could be established as in agreement with his plants the fact would bear directly on the problem of the origin of *O. Lamarckiana*. The flowers are large and the buds rather stout as we find them in our cultivated *Lamarckiana*, but the sepal tips are longer and the bracts much narrower than in *Lamarckiana*. The most striking characters of this specimen as shown in the photograph are the narrow lanceolate leaves and the extraordinary length of their petioles. That such a plant could be related to De Vries's *Lamarckiana* which has ovate-lanceolate leaves, sessile or almost sessile, seems to me well nigh impossible. Of the pubescence De Vries tells us nothing, yet the numerous buds on the specimen should make it easy to determine this character and it may become a crucial point in judging the possible or impossible relationships of the plant.

In the discussion which must develop from the conclusions of De Vries he has taken by far the more difficult position since he attempts an identification of herbarium material with a type very accurately known to us through widely cultivated living forms. My argument is presented primarily against his identifications. It is not in any degree necessary to my argument that I should assign the sheets under consideration to definite species. Whether this can be done for any of them time will tell and I must repeat that as evidence the character of the pubescence may prove of the greatest value. I am working on the hypothesis that the specimens of Lamarck and that of Abbé Pourret are forms of *O. grandiflora* Solander. As for the specimen of André Michaux, so many remarkable forms of *Œnothera* are coming into the experimental garden from the southern and western United States that I am quite unwilling to express at present even a guess as to its affinities.

De Vries has welcomed my suggestion that the source of the cultures of Carter and Company may have been not Texas, as they state, but England. This possibility seems to me to offer an important line of investigation of early British records and collections, but at present the suggestion appears to me nothing more than a working hypothesis, although well worthy of attention. Texas and the West have some wonderful large-flowered œnotheras and Carter and Company may have obtained from such sources a plant which later hybridizing with other forms produced the *Lamarckiana* of our present cultures. That there are American western species which will hybridize with European *biennis* and produce a synthetic *Lamarckiana* is I believe established by my present studies with *Œnothera franciscana* Bartlett.

In recent papers I have reported that first generation hybrids of *O. franciscana* pollinated by the Dutch *biennis* have the essential taxonomic characters of the small-flowered forms of *O. Lamarckiana.* They differ from *Lamarckiana* in relatively small plus or minus expressions of these characters. It was to be expected that large F_2 generations would give a wide range of variation or segregation of characters and that forms would appear much closer to *Lamarckiana* than the parent F_1 plants. This proved to be the case in F_2 cultures of last summer (1914) totaling about 1,600 plants. Among these I obtained a number of individuals which were so close to the large-flowered *Lamarckiana* that flowering shoots could scarcely be distinguished as to

pubescence, foliage, inflorescence, buds, flowers, and capsules. The rosettes were also *Lamarckiana*-like. Only in habit was it somewhat difficult to match the symmetry of *Lamarckiana*. That further selection in later generations is likely still to further improve on the results of this synthesis seems altogether probable. These studies will shortly be described in full.

I am well aware that a synthesis of a *Lamarckiana*-like hybrid even should it throw in successive generations a series of marked variants (mutants) will not be considered by De Vries and his disciples as casting doubt on the validity of the "mutation" of *Lamarckiana*. They will say that in this case the hybrid took its mutating habit from one or both of the parents. Since in my cross one of the parents is the Dutch *biennis* which Stomps has shown can produce *nanella, semi-gigas* and *sulfurea* mutants, it will be claimed that any behavior of my hybrids similar to mutation will be due not to the mixing of diverse germ plasms, *i. e.*, to crossing, but will be merely a further expression of mutating habits inherent in the germ plasm of at least *biennis* if not also of *franciscana*.

This phase of the discussion may rest until we know the future behavior of my hybrids and the possibilities of the Dutch *biennis* as a form capable of mutation. It is to be expected that Stomps will carry out his very important studies on a scale that will virtually exhaust the mutative possibilities of this species. Such a study on a close-pollinated species of *Œnothera* so well known as the Dutch *biennis* will give, it seems to me, the safest data that has yet been published by students of mutation among the *Œnotheras*. It becomes a matter of great interest to know the range of variants that such a type can produce. Similar studies among some of the wild American species should also be made. The open-pollinated assemblage of forms to which *Lamarckiana* belongs must always be open to suspicion of hybridization more or less remote in time or distant in relationship. Only prolonged experiment can establish an open-pollinated *Œnothera* as free from the taint of crossing.

It is, I trust, clear that one may believe very strongly that *Œnothera Lamarckiana* is not safe material on which to base experiments designed to test the mutation theory and yet remain receptive to evidence that may come from other sources.

BRADLEY MOORE DAVIS

UNIVERSITY OF PENNSYLVANIA,
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CYCLES AND RHYTHMS AND THE PROBLEM OF "IMMORTALITY" IN PARAMECIUM

PROFESSOR GARY N. CALKINS

Columbia University

The recent brilliant work of Woodruff and Erdmann has thrown a flash of light upon the old question of age and death in protozoa and upon the problem of the significance of conjugation. The long, successful cultivation of *Paramecium aurelia* by Woodruff led Jennings to say:

> The work of Woodruff demonstrates that the very limited periods within which Maupas and Calkins observed degeneration has no significance for the question as to whether degeneration is an inevitable result of continued reproduction without conjugation. In other words, it annihilates all the positive evidence for such degeneration, drawn from work on the infusoria. It justifies the statement that the evidence is in favor of the power of these organisms to live indefinitely, if they are kept under healthful conditions. It shows that Weismann was correct in what he meant by speaking of the potential immortality of these organisms.[1]

The same work of Woodruff led Minot to say in the course of his German lectures:

> Quite conclusive as to the absence of senescence are the experiments of L. L. Woodruff, who has maintained a pedigreed race of *Paramecium* for five years without conjugation.[2]

Woodruff also makes the statement in different publications that the cells of his pedigreed race of *Paramecium*

[1] "Age, Death and Conjugation in the Light of Work on Lower Organisms," *Pop. Sci. Mo.*, June, 1912, p. 568.

[2] "Modern Problems of Biology," 1913, p. 62.

aurelia possess the potentiality to perpetuate themselves indefinitely by division under proper environmental conditions. In short, his results have given almost the only experimental evidence in support of the view, advocated by Weismann, that protozoa are potentially immortal.

The importance of this generalization and the deductions from it are self evident, and it is unfortunate that so many should have advanced it before the life history of *Paramecium aurelia* was fully known. Woodruff is to be congratulated, however, in that he, with Miss Erdmann, has now worked out stages in the life history of this organism which go far in clearing up the discrepancy between his results and those obtained by Maupas and his followers.

Woodruff has carried on his pedigreed race of *Paramecium aurelia* for more than seven years with a fairly uniform division rate, subject, however, to occasional and periodic fluctuations which he calls rhythms. These correspond roughly to what I have termed cycles which end in depression periods and, unless stimulated, by death; in rhythms, however, Woodruff maintains, there is no evidence of depression.[3] Recently Woodruff finds from a careful study of material fixed during the low periods of his division rate rhythms that there takes place a complete nuclear reorganization, after which the organisms continue to live with renewed vitality as shown by the ascending division rate. This process consists in the disintegration and probable absorption in the cytoplasm of the old macronucleus, one or more divisions each of the old micronuclei, degeneration of some of the products of these divisions, and the ultimate reformation of functional macronuclei and micronuclei from others. These are, in essence, the important new facts which cytological study has revealed in the life history of *Paramecium aurelia,* and some evi-

[3] I would like to suggest to Professor Woodruff that he work out the death rate, the data for which he can undoubtedly obtain from his records. I venture to predict he will find that the death rate rises with the decline of the division rate and during the low sweep of the rhythms.

dence is further adduced to show that similar processes occur in *Paramecium caudatum.*

While there is little to criticize in regard to the facts as described for this remarkable process, there is room for difference of opinion in regard to the conclusions which Woodruff and Erdmann draw from them. In addition to what Hertwig has already written about this work, I would call attention particularly to their conclusions concerning endomixis, parthenogenesis, conjugating and nonconjugating lines, the life cycle, and the potential immortality of *Paramecium.*

In regard to endomixis the authors state:

Since the process results in the dissemination of the material from the old macronucleus and the so-called reduction micronuclei in the cell, it gives the opportunity for a rearrangement of the molecular constitution of the cell. This involves a more profound intermingling of nuclear and cytoplasmic substances than is possible during the typical vegetative life of the cell. *Since this intermingling occurs within a cell we term this reorganization process endomixis.* Endomixis is followed by a slight acceleration of cell phenomena and a new rhythm is initiated.[4]

Further on they add:

We would therefore put emphasis on molecular rearrangement as the result common both to endomixis and to conjugation.[5]

Nearly forty years ago Engelmann interpreted conjugation in much the same way as a process of reorganization of the cell:

. . . die Conjugation der Infusorien leitet nicht zu einer Fortpflanzung durch 'Eier,' 'Embryonalkugeln' oder irgend welche andere Keime, sondern zu einem eigenthümlichen Entwickelungsprocess der conjugirten Individuen, den man als Reorganization bezeichnen kann.[6]

In several places in the same publication Engelmann speaks of physical and chemical changes as accompanying

[4] Woodruff and Erdmann, "A Normal Periodic Reorganization Process without Cell Fusion in Paramecium," *Jour. Exp. Zool.*, Vol. 17, No. 4, 1914, p. 491. The italics are in the original.

[5] *Ibid*, p. 491.

[6] "Ueber Entwickelung und Fortflanzung von Infusorien," *Morph. Jahrb.*, 1, 1876, p. 628.

conjugation. A similar interpretation was given by Calkins:

> . . . it is now a well-known fact that in this process of reorganization the old macronucleus fragments and ultimately disappears in the cytoplasm. This disappearance must give rise to a great increase in the nucleo-protein content of the cell, therefore to a new chemical composition of the cell as a whole. We have recently shown that, under certain conditions, nucleo-proteins (especially the purines) have a markedly stimulating effect on the rate of cell division.[7]

Now such intermingling is no more characteristic of this process of asexual reorganization than it is of the reorganization following conjugation. In both cases, as Woodruff and Erdmann show, reorganization is effected by the physical and chemical change of the old macronucleus and portions of the old micronucleus or micronuclei. The sole difference in these processes of reorganization is not to be found in the molecular rearrangement of the cell, but, as Woodruff and Erdmann state, in the presence after conjugation of a syncaryon and the nuclei derived from it. This difference, however, does not amount to much in closely related pairs in conjugation. Several observers have shown that closely related individuals, even sister cells, of *Paramecium* may conjugate, and I have followed out through 360 generations the history of such an endogamous exconjugant from a pair which came from the same ancestral cell not more than ten days prior to conjugation. There can not be a great difference in the syncaryon resulting from such a union, over the functional micronucleus had it undergone asexual endomixis. In other words, the excellent term endomixis does not indicate phenomena peculiar to asexual reorganization in *Paramecium*, but applies equally well to the process of reorganization following conjugation. The terms asexual endomixis and sexual endomixis may serve to distinguish the process of intermingling during parthenogenesis and after conjugation, respectively.

[7] "The Paedogamous Conjugation of *Blepharisma undulans*," *Jour. Morph.* Vol. 23, 1912, p. 685.

Woodruff and Erdmann limit the application of the term endomixis to the process of reorganization without conjugation:

We therefore have employed a new term "endomixis" for the reorganization process in Paramecium, in preference to parthenogenesis which Hertwig applied when he incidentally noted some isolated stages of the nuclear phenomena which we have elucidated.[8]

A new name can not alter the significance of a process or phenomenon. Parthenogenesis, in its broad sense, is the development of an individual from an egg without fertilization. In the same sense that a *Paramecium* ex-conjugant develops into a new individual, so does a *Paramecium* after this process termed endomixis. Woodruff and Erdmann say:

In parthenogenesis there is a chromatin reduction which occurs and is compensated for either in the egg itself or in some later period of the life cycle of the race.[9]

The authors are not very happy in selecting this feature as distinguishing parthenogenesis from asexual endomixis, for in most cases of recognized parthenogenesis in metazoa chromatin reduction plays no part; for example, the majority of parthenogenetic eggs give off only one polar body, thus retaining in the egg the diploid number of chromosomes; others, notably the aphids and phylloxerans, do not undergo synapsis or chromatin reduction; some others it is true, give off both polar bodies and develop with the haploid number of chromosomes as is the case in bees (males), and in artificial parthenogenesis. As to the significance of parthenogenesis neither polar body formation nor chromosome reduction furnishes the key, for in many cases the eggs are predestined to parthenogenetic development long before the polar body nuclei are formed.

In regard to the reducing divisions of the chromosomes in *Paramecium* we know very little. Evidence has been adduced to indicate that the chromosomes are divided

[8] *Ibid.*, p. 493.
[9] *Ibid.*, p. 492.

longitudinally in both the first and the second divisions of the maturation process. The significance of the third division is as obscure in *Paramecium* as maturation is in some metazoan hermaphrodites.

In parthenogenesis, finally, we are dealing with a biological phenomenon, not with an interpretation of parthenogenesis by Winkler or Strasburger or any other individual, and to interpret this highly significant phenomenon in *Paramecium* solely in the light of such definitions, as Woodruff and Erdmann do (p. 493), does not carry conviction, nor does it conceal the real significance of the phenomenon. Asexual endomixis in *Paramecium* is parthenogenesis and nothing else, as Hertwig originally maintained in connection with these same phenomena. Nor, except for the protozoa, is it a "new type of parthenogenesis" for, if we accept conjugation as equivalent to fertilization, its analogue is shown by the majority of parthenogenetic eggs.

In regard to conjugating and non-conjugating races of *Paramecium*, Woodruff and Erdmann state:

> *Thus it is proved that both the reorganization process and conjugation are potentialities of the same race*—and therefore there is no evidence for the view of Calkins ('13) that conjugating and non-conjugating races of Paramecium exist, or that "apparently some paramecia are potential germ cells, others are not."[10]

This is rather a sweeping generalization to draw from one pedigreed line in which conjugating animals appeared only after six years in culture. If every *Paramecium* is a potential germ cell, why was it that no pairs of conjugating *aurelia* were found during these six years? Or, in Calkins and Gregory's observations on the first 32 cells and the pure lines arising from them, all from a single ex-conjugant, why was it that all lines from one quadrant gave epidemics of conjugation whenever the test was made during a period of six months, while all other lines from the remaining three quadrants failed to give a single pair when tested under identical conditions?

[10] *Ibid.*, p. 490. Italics in the original.

Woodruff and Erdmann maintain that "under just the proper conditions" conjugation will occur; this, of course, can not be denied, but the fact that under the same conditions some lines will conjugate while others will not shows a physiological difference between them which can not be gainsaid. I have no paternal jealousy whatsoever in regard to the terms "conjugating lines" and "non-conjugating lines," and am entirely willing to accept in their place any terms which indicate the physiological difference that I wished to express. I know of no terms that express the conditions adequately. Substitute for them, if more suitable, such expressions as "always ready to conjugate" and "rarely ready to conjugate." Our observations on the 32 lines certainly justify the statement that some lines in regard to conjugation, were always ready, while others were rarely ready. Woodruff and Erdmann have paid no attention to the physiological conditions which the (perhaps unfortunate) expressions "conjugating lines" and "non-conjugating lines" were meant to express. It is true that after ten months all but four of the so-called non-conjugating lines each furnished a few pairs of conjugating individuals, just as Woodruff's line did after six years, facts which show that the terms "conjugating lines" and "non-conjugating lines" as applied to races of *Paramecium,* if used at all, should be used only in respect to relative intensity of conjugating power. In this sense Woodruff's race is a non-conjugating race. We have found, furthermore, that conjugating lines have a lower vitality as measured by the division rate, and a much higher death rate, than do non-conjugating lines, all but four of the eight lines from the conjugating quadrant dying out within three months as against four of the twenty-four lines of the non-conjugating quadrants, while at the end of twenty months only one conjugating line was alive and sixteen non-conjugating lines, a mortality of 87.5 per cent. for the former and 33.3 per cent. for the latter. In *Paramecium* it is conceivable that lines with a high conjugating

power have a less well developed power of asexual endomixis than do lines that are relatively sterile, and this, correlated with their reduced vitality, if conjugation were prevented, would account for the death of all pedigreed races prior to Woodruff's, which, as Woodruff and Erdmann now show, has a high power of asexual endomixis. We are still justified, I believe, in maintaining the statement—modified now by their description of asexual reorganization—as quoted by Woodruff and Erdmann:

> Woodruff's *Paramecium aurelia* is evidently a *Paramecium Methuselah* belonging to a non-conjugating line the life history of which is not known in any case.[11]

> It is clear that the cycle emphasized by Maupas, Calkins and others is merely a phantom which has continually receded as each successive investigator has approached the problem with improved culture methods until it has vanished with Woodruff's race of (so far) 4,500 generations. What remains then is the rhythm and in the light of the present study, which demonstrates the underlying cytological phenomena of which it is an outward physiological expression, the whole problem takes on a new aspect. The cell automatically reorganizes itself periodically by a process which, in its main features, simulates conjugation—but without a contribution of nuclear material from another cell. Therefore it is evident (as has been shown by this culture) that the formation of a syncaryon, whose components are derived from two cells, is not necessary for the continued life of the cell—it has an internal regulating phenomenon which is entirely adequate to keep it indefinitely in a perfectly normal condition.[12]

Here we are brought up sharply to face the question which every student of pedigreed infusoria since Maupas has tried to solve. Woodruff and Erdmann conclude from their observations that old age and natural death do not occur in *Paramecium* and that the so-called "cycle" is non-existent. I would draw from their observations exactly the opposite conclusions, viz., that the one apparent exception among pedigreed races, to the rule of depression and natural death in the absence of conjugation or its equivalent, is now removed, and that Woodruff's culture is no more than a long series of cycles.

[11] *Ibid.*, p. 429.

[12] Woodruff and Erdmann, p. 489.

We understand by a "cycle," in the sense with which the term was first employed by Calkins, a more or less periodic alternation of high and low vitality as measured by the division rate. The lowering division rate indicates the approach of a period of depression which was interpreted as the equivalent of old age in metazoa, since it indicates a weakening in the chain of vital activities and ends in death unless conjugation or its equivalent is permitted. No one since Maupas, so far as I am aware, has attempted to limit a cycle in terms of definite numbers of generations or definite lengths of time. In 1904 I stated:

> The well-marked cycles, therefore, with periods of depression which demanded stimulation of a decided character, were apparently of *six months* duration, while intermediate cycles of less importance were about three months long. . . . During the first three cycles the number of generations was nearly the same (200, 198, and 193, respectively), the last, on the other hand, was much less, the individuals dividing only 126 times.[13]

The period of six months, more or less, or 200 ± generations were not regarded as measures of the cycle, and it was understood at that time that conjugation or its equivalent always inaugurates a new cycle. Woodruff in 1905 introduced the term "rhythm" to designate the lesser periodic fluctuations which I had called "intermediate cycles." Since the entire substance of the much-discussed problem of immortality in infusoria is bound up with this question of the cycle, it is necessary to analyze the so-called rhythms of Woodruff to see how they agree with or differ from the so-called cycles. In *Paramecium* the cycle consists of the history of a bit of protoplasm in an ex-conjugant and its progeny from which conjugation or its equivalent is excluded, until natural death of the entire race ensues. If conjugation or its equivalent occurs the old cycle is abandoned and a new one is started, and there must be as many new cycles as there are times when conjugation or its equivalent takes place. It is imma-

[13] "Studies on the Life History of the Protozoa," IV. *Jour. Exp. Zool.*, Vol. I, 1904, p. 424.

terial, furthermore, whether such conjugation occurs between individuals of the same race, or between individuals of diverse ancestry, the effect is the same in putting off ultimate weakness and death. With repeated conjugations in such a race the ultimate death may be postponed indefinitely, and this was the argument on which Weismann's revised theory of potential immortality was based.

Now it is exactly the same with Woodruff's rhythms. He finds in his long culture repeated instances of ascending and descending division rates in fairly regular alternate succession. The descending division rate is stopped by an "internal regulatory phenomenon, endomixis."[14] Woodruff and Erdmann, while showing that endomixis is different from conjugation in the absence of a syncaryon, apparently accept it as equivalent to conjugation in connection with vitality of the protoplasm:

> Endomixis and conjugation may occur simultaneously in different animals of the same culture, thus strongly suggesting that the same *general* conditions lead to both phenomena—one animal meeting the conditions one way and another by the other, and that *both phenomena fill essentially the same place in the economy of life of Paramecium aurelia.*[15]

Again they say:

> Endomixis does initiate a new rhythm in the life history of Paramecium, *i. e.*, a period of increased metabolic activity and therefore of reproductive activity, and since its fundamental morphological features are almost identical with those preliminary to the formation of the stationary and migratory micronuclei in conjugation, it lends strong support to the view that the dynamic aspect of conjugation is not absent.[16]

Throughout the long period of seven years the *Paramecium aurelia* protoplasm without conjugation: "has undergone endomixis frequently, undoubtedly on the average once each month" (*ibid.*, p. 495). Hertwig has already shown, as I do above, that asexual endomixis is parthenogenesis, and if, in connection with the problem of vitality, this is equivalent to conjugation, then we are

[14] *Ibid.*, p. 497.

[15] *Ibid.*, p. 492; the italics at the end are mine.

[16] *Ibid.*, p. 496.

justified in saying that throughout the seven years Woodruff's *Paramecium* has undergone the equivalent of conjugation on the average once each month, and if it is equivalent to conjugation, then his long culture of more than 4500 generations has no bearing on the question of old age and natural death in *Paramecium.*

Nothing in this work of Woodruff and Erdmann seems more clearly and forcibly demonstrated than that the cycle, this "phantom" of many investigators, resolves itself into a demonstrated fact, and that Woodruff's "rhythm" and Calkins's "cycle" are but different names for the same phenomenon. If natural death is a necessary end to justify our use of the term "cycle," we may ask the pertinent question: What happened to those individuals which did not undergo asexual endomixis in Woodruff's long culture? If they died, does not this fact indicate the end of a cycle? If they underwent parthenogenesis, the equivalent of conjugation, does not this fact indicate the beginnings of new cycles? If they continued to live without reorganization, evidence for which has never been given by Woodruff, then there would be some justification for our authors' conclusion. To argue that it is the same race which continues after asexual endomixis is to use the same argument that Weismann used unsuccessfully, viz., that an ex-conjugant is the same old individual since no corpse has been formed and therefore the infusoria are immortal.

The frequent statement made by Woodruff that his long culture sustains the view that old age and need of conjugation are not necessary attributes of living matter are contradicted by these later results. For example, he states in 1913:

Diese Untersuchung hat uns gezeigt, dass, unter günstigen äusseren Umständen, das Protoplasma der zuerst isolierten Zelle mindestens die Potenz hatte, ähnliche Zellen bis zu einer Zahl von 2^{3340} und eine Masse Protoplasma von mehr als 10^{1000} mal der Masse des Erdballes zu erzeugen. Dieses Resultat, glaube ich, bestätigt unzweifelhaft die Annahme, dass das Protoplasma einer einzigen Zelle unter günstigen

äusseren Umständen ohne Hilfe von Konjugation oder einer künstlichen Reizung imstande ist, sich unbegrenzt fortzupflanzen und zeigt ferner in klarer Weise, dass das Altern und das Befruchtungsbedürfnis nicht Grundeigenschaften der lebendigen Substanz sind.[17]

I am entirely in sympathy with Hertwig when he says, in connection with this citation:

Nach meiner Ansicht sind die Resultate, zu denen in den unseren Auseinandersetzungen zum Ausgangspunkt dienenden Artikel Woodruff gemeinsam mit Rhoda Erdmann gelangt ist, mit den hier zitierten Sätzen unvereinbar.[18]

The discovery of parthenogenesis in the life cycle of *Paramecium aurelia* by Woodruff and Erdmann clears up the obscurity which has involved all theoretical discussions following pedigreed culture work with infusoria, and we now see with much clearer vision the probability, first, that conjugation or its equivalent has primarily the result, as originally interpreted by Bütschli, of offsetting and overcoming the progressive weakening of vitality in infusoria; second, that more or less definite cycles of vigor and depression, ending in natural death unless conjugation or its equivalent supervenes, are characteristic of all pedigreed races of infusoria; third, that physical "immortality" is true of *Paramecium* and other ciliates only in the same sense that it is true of metazoa; fourth and last, that *Paramecium* protoplasm is subject to the same laws of physiological usury that apply to metazoa, and undergoes phenomena which, in metazoa, we call old age, and which, as in metazoa, ends in natural death unless conjugation, or its equivalent parthenogenesis, saves the race.

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[17] Dreitausend und dreihundert Generationen von *Paramecium* u. s. w.," *Biol. Centr.*, Vol. 33, No. 1, 1913, p. 35.

[18] "Ueber Parthenogenesis der Infusorien," etc., *Biol. Centr.*, Vol. 34, No. 9, 1914, p. 577.

THE PHENOMENON OF SELF-STERILITY[1]

PROFESSOR E. M. EAST

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In both animals and plants in which the two sexes have been combined in the same individual, cases have been found where self-fertilization is practically impossible. This gametic incompatibility has been called self-sterility, although the term is hardly proper as applied to normal functional gametes that may fuse with their complements in the regular manner, provided each member of a pair has been matured in a separate individual.

In plants the phenomenon has been known since the middle of the nineteenth century, in animals a corresponding discovery was made in 1896 by Castle, the species being one of the Ascidians, *Ciona intestinalis.* During the eighteen years that have passed since Castle's discovery, *Ciona* has been studied on a large scale by Morgan (1905), Adkins (Morgan, 1913), and Fuchs (1914). The botanists, however, have lagged somewhat behind; for, in spite of having been acquainted with self-sterility in plants for over half a century, and having found over thirty species where a greater or less degree of self-sterility occurs from which to select material, very few thorough investigations into the physiology of the subject have appeared.

The main facts regarding fertilization in *Ciona intestinalis* are about as follows:

1. Under uniform suitable conditions, individuals vary in degree of self-sterility, it being exceptional to find an animal that is perfectly self-sterile.

2. Self-fertility has never equaled cross-fertility, though the possibility remains that some animals may be self-

[1] Read by title at the thirty-second meeting of the American Society of Naturalists, December 31, 1914.

fertilized as easily as they may be crossed with certain particular individuals.

3. The ease with which the ova of any animal "*A*" may be fertilized by the sperm of other individuals may vary.

Morgan (1913) concluded from his own work and that of Adkins that there were wide differences in the compatibility of ova to different sperm. Fuchs (1914) maintained that 100 per cent. of segmenting eggs can be obtained in every cross if the ova are normal and a sufficiently concentrated sperm suspension is used. It is possible that Fuchs is correct and that varying concentrations of sperm suspension were the cause of Morgan's and Adkins's results, yet the possibility of differences in this regard inherent in the individual is not to be overlooked. It will be seen later that I regard the matter as of great importance to the general subject.

4. A chemical basis for self-sterility is shown in Fuch's experiments by (*a*) the decrease in ease of cross-fertilization after contact of ova with sperm from the same animal, and by (*b*) the difference in ease of self-fertilization after various artificial changes in the chemical equilibrium of the medium surrounding the ova.

From the botanical side various studies on the physiology of self-sterility have appeared since such investigations were initiated by Hildebrand in 1866. At this time it is necessary for us to consider only those of Jost (1907), Correns (1912), and Compton (1913).

Jost was able to show that in self-sterile plants tubes formed from their own pollen were so limited in their development that fertilization did not occur, although the necessary length of pollen tube was easily developed after a cross-fertilization. He saw as the cause of these phenomena the presence of "individueller Stoffe." Pollen was indifferent to "Individualstoff" of the same plant, but was stimulated by that of other plants.

Correns (1912), working with one of the bitter cresses, *Cardamine pratensis,* obtained results to which he gave a simpler interpretation. Starting with two plants, *B* and

G, he crossed them reciprocally and tested 60 of the offspring by pollinating from the parents, on the parents, and *inter se.* The back crosses of $(B \times G)$ or $(G \times B)$ with *B* and with *G* apparently indicated four classes about equal in size with reference to gametic compatibility: (1) plants fertile with both *B* and *G*; (2) plants fertile with *B* but not with *G*; (3) plants fertile with *G* but not with *B*; (4) plants fertile with neither *B* nor *G*.

To these facts Correns gave a Mendelian interpretation by assuming the existence of two factors each of which inhibits the growth of pollen tubes from like gametes. Representing these factors by the letters *B* and *G*, it is clear that types *BB* and *GG* could never be formed. The original plants were supposed to be of classes *Bb* and *Gg*, respectively. When crossed there resulted the four types *BG, Bg, bG* and *bg*. Plants of types *BG, Bg,* and *bG* should be self-sterile, while plants of the type *bg* should be self-fertile. Plants *BG* should be fertile with plants *bg*, plants *Bg* should be fertile with *bG* and *bg*, and plants *bG* should be fertile with *Bg* and *bg*. As a matter of fact Correns's results were not clearly in accord with the theory. Plants of the type *bg* were not self-fertile, and the other classes of matings showed many discrepancies. It is only fair to say, however, that the author recognized some of these difficulties, but believed them to be due to other inhibitors.

In a part of Compton's (1913) work, a still simpler interpretation of self-sterility is offered, at least for a particular case, that of *Reseda odorata.* Darwin's original discovery that both self-sterile and self-fertile races of this plant exist was confirmed and the following results obtained in crossing experiments. Self-sterile plants crossed either with self-sterile or with self-fertile plants gave only self-sterile offspring. Certain self-fertile plants, however, gave only self-sterile offspring when self-pollinated. Other self-fertile plants gave ratios of 3 self-fertile to 1 self-fertile offspring when self-pollinated, and ratios of 1:1 when crossed with pollen from self-sterile

plants. For these reasons he regards self-fertility as a simple Mendelian dominant to self-sterility in the case studied. I believe Compton would draw no such sharp line about self-sterility in general. In fact, he follows Jost in suggesting the presence of a diffusible substance in the tissues of the style and stigma which retards or promotes pollen tube growth after self-pollination or cross-pollination in some manner analogous to the mechanism that promotes animal immunity or susceptibility after infection.

The only alternative general hypothesis has been proposed by Morgan, and this can be discussed more advantageously after the presentation of my own work, of which only an abstract will be given at this time.

In 1909 I made a cross between a small red-flowered *Nicotiana, Nicotiana forgetiana* (Hort.) Sand. and the large white-flowered *Nicotiana* of the garden *Nicotiana alata* Lk. and Otto. var. *grandiflora* Comes. All of the plants of the F_1 generation appeared to be self-sterile. Tests of *Nicotiana forgetiana*[2] have shown these plants also to be self-sterile, but both self-fertile and self-sterile plants of the other parent have been found. From data gathered later, there seems to be no doubt that a self-sterile plant of *Nicotiana alata grandiflora* was used in the actual cross. This conclusion seems reasonable in view of the fact that of over 500 plants of the F_1, F_2, F_3 and F_4 generations tested, not a single self-fertile plant was found.

The plants of the F_1 generation were all vigorous and healthy, and in spite of the fact that they resulted from a species cross which Jeffrey claims always produces large amounts of abnormal pollen, a large number of examinations of pollen from different individuals showed from 90

[2] I thought originally that both of these species (East, 1913) were self-fertile. Seed had been obtained from a carefully bagged inflorescence of each species in 1909. Either the plant of *N. forgetiana* which gave this seed was self-fertile—something that I have never been able to find since that time—or there was an error in manipulation. At any rate, the plants resulting from this seed were all self-sterile.

to 100 per cent. of morphologically perfect pollen grains, a condition about the same as was found in the pure species. To this statement there is one exception. A single plant was found with only about 2 per cent. of good sound pollen.

Several experiments were made in which crossing and selfing was done on a large scale, using plants of the F_2, F_3 and F_4 generations which had segregated markedly in size and were of at least 8 different shades of color. In one of these experiments 20 plants of the F_2 generation coming from 2 crosses of F_1 plants were used. It was planned to make all possible combinations of these plants, 400 in all. This task proved overburdensome, however, and in addition to the self-pollinations but 131 inter-crosses were made with the following results.

1. Each plant was absolutely self-sterile.

2. Leaving out of consideration the plant with shrunken imperfect pollen only two crosses failed. This failure of 1.5 per cent. of the crosses may have been due to improper conditions at the time of the attempts, but as a number of trials were made the possibility remains that there is a small percentage of true cross-sterility.

3. Of the 129 successful inter-crosses, 4 produced capsules with less than 50 per cent. of the ovules fertilized. The remaining crosses produced full capsules. It is barely possible that this result shows a slight variability in ease of cross-fertilization, but I am more inclined to believe that these 4 cases where a low percentage of fertilized ovules were obtained were accidental.

Other crossing experiments of the same kind have corroborated these results. Out of 120 inter-crosses, only 3 failed.

Later, something over 100 inter-crosses were made between 12 plants of an F_3 population resulting from crossing two sister F_2 plants. Six of the attempts at cross-fertilization—3 to 8 trials per plant being made—were failures. These plants as well as others tested were com-

pletely self-sterile, and apparently there was cross-sterility in about 6 per cent. of the possible combinations.

In the F_4 generation, 10 plants resulting from crossing two sisters of the F_3 generation were selected for experiment. Unfortunately, I was able to make only 58 intercrosses, 5 of which, almost 10 per cent., failed.

Back crosses have furnished another line of experiment, though they have not been carried on as systematically as were those of Correns. Nearly 85 back-crosses using plants from the progeny of four combinations which included four individuals as parents, have been made. The plants themselves all proved self-sterile, and in addition 5 of the back crosses failed.

When these experiments were begun I expected to find that the facts would accord with a simple dihybrid Mendelian formula similar to that which Correns later proposed as an interpretation of his results, yet only by considerable stretching and a vivid imagination will Correns's data fit such an hypothesis, and my own data do not fit at all. No self-fertile plants have been produced by any combination, and cross-sterility is a possibility in only from 1.5 to 10 per cent. of the combinations. Furthermore, Correns's idea of inhibitors appears unlikely from some other data I have gathered with the help of Mr. J. B. Park. Ten plants were involved in this experiment. Pairs of plants were provided to furnish series of selfed and crossed flowers. The pistils of these flowers were fixed at regular periods after pollination, stained, sectioned, and the pollen tubes examined. Fertilization not later than the fourth day marked the end point of the crossed series, the dropping of the flowers between the eighth and the eleventh day ended the selfed series. As the flowers on each plant had about the same length pistils, curves of pollen tube development for both crossing and selfing could be constructed. The pollen grains germinated perfectly on stigmas from the same plant, from 1,200 to 2,000 tubes having been counted in sections of single pistils. The difference between the development

of the tubes in the selfed and the crossed styles is wholly one of rate of growth. The tubes in the selfed pistils develop steadily at a rate of about 3 millimeters per twenty-four hours. There is even a slight acceleration of this rate as the tubes progress. If the flowers were of an everlasting nature one could hardly doubt but that the tubes would ultimately reach the ovules, though this would not necessarily mean that fertilization must occur. Since the maximum life of the flower is about 11 days, however, the tubes never traverse over one half of the distance to the ovary. On the other hand, the tubes in the crossed pistils, though starting to grow at the same rate as the others, pass down the style faster and faster, until they reach the ovary in four days or less.

From these facts it seems reasonable to conclude that the secretions in the style offer a stimulus to pollen tubes from other plants rather than an impediment to the development of tubes from the same plant.

The whole question, therefore, becomes a mathematical one, that of satisfying conditions whereby no stimulus is offered to pollen tubes from the same plant, but a positive stimulus is offered to tubes from nearly every other plant.

Morgan has given an answer to this question in a general way. If I understand his position correctly, my own conclusions are not very different from his, but are somewhat more definite. Morgan (1913) states that the results of Adkins and himself on *Ciona intestinalis* can best be understood by the following hypothesis:

> The failure to self-fertilize, which is the main problem, would seem to be due to the similarity in the hereditary factors carried by the eggs and sperm; but in the sperm, at least, reduction division has taken place prior to fertilization, and therefore unless each animal was homozygous (which from the nature of the case cannot be assumed possible) the failure to fertilize can not be due to homozygosity. But both sperm and eggs have developed under the influence of the total or duplex number of hereditary factors; hence they are alike, *i. e.*, their protoplasmic substance has been under the same influences. In this sense, the case is like that of stock that has long been inbred, and has come to have nearly the same hereditary complex. If this similarity decreases

the chances of combination between sperm and eggs we can interpret the results.

I make this quotation to show Morgan's viewpoint. It is for him to say whether the following conclusions are extensions of his own or not.

The tolerably constant rate of growth of pollen tubes in the pistils of selfed flowers, compared with the great acceleration of growth of the tubes from the pollen of other plants as they penetrate nearer and nearer to the ovary, undoubtedly shows the presence of stimulants of great specificity akin to the "Individualstoffe" of Jost. We are wholly ignorant of the nature of these stimulants, but I am inclined towards a hypothesis differing somewhat from his. Experiments by several botanists, which I have been able partially to corroborate, point to a single sugar, probably of the hexose group, as the direct stimulant. The specific "Individualstoffe" I believe to reside in the pollen grains and to be in the nature of enzymes of slightly different character, all of which, except the one produced by the plant itself for the use of its own pollen or by other plants of identical germinal constitutions, can call forth secretion of the sugar that gives the direct stimulus. At least this idea links together logically the fact of the single direct stimulus and the need of "Individualstoffe" to account for the results of the crossing and selfing experiments. But whether or not this be the correct physiological inference, the crossing and selfing experiments call for a hypothesis that will account for no stimulation being offered the tubes from the plant's own pollen, while at the same time great stimulation is given the tubes from the pollen of nearly every other plant.

This is a straight mathematical problem, and it is hardly necessary to say that it is insoluble by a strict Mendelian notation such as Correns sought to give. This is obvious to any one familiar with the basic mathematics of Mendelism. On the other hand, a near Mendelian interpretation satisfies every fact.

Let us assume that different hereditary complexes stim-

ulate pollen tube growth and in all likelihood promote fertilization, and that like hereditary complexes are without such effect. One may then imagine any degree of heterozygosis in a mother plant and therefore any degree of dissimilarity between the gametes it produces, without there being the possibility of a single gamete having anything in its constitution not possessed by the somatic tissues of the mother plant. From the chromosome standpoint of heredity the cells of the mother plant are duplex in their organization; they contain N pairs. The cells of the gametes contain N chromosomes, one coming from each pair of the mother cell; but they are all parts of the mother cell and contain nothing that that cell did not contain. These gametic cells can not reach the ovaries of flowers on the same plant because they can not provoke the secretion of the direct stimulant from the somatic cells of that plant.

All gametes having in their hereditary constitution something different from that of the cells of a mother plant, however, can provoke the proper secretion to stimulate pollen tube growth, reach the ovary before the flower wilts and produce seeds. Such differences would be very numerous in a self-sterile species where cross-fertilization must take place; nevertheless like hereditary complexes in different plants should be found, and this should account for the small percentage of cross-sterility actually obtained. It must be granted that this hypothesis satisfies the facts, but that is not all. It is admittedly a perfectly formal interpretation, but from a mathematical standpoint,—granting the generality of Mendelian inheritance,—it is the only hypothesis possible that can satisfy the facts.

Let us now look into a few of the ramifications of the subject. Examinations of the pistils that have been sectioned after cross-pollination show a considerable variation in the rate of growth of individual pollen tubes, though our curves of growth have been made by taking the average rate of elongation. Is this variation a result

of chance altogether or must we assume a differential rate of growth increasing directly with the constitutional differences existing between the somatic cells and the various gametes? If we assume that any constitutional difference between the two calls forth the secretion of the direct stimulus to growth, chance fertilization by gametes of every type different from that of the mother plant will ensue; if there is a differential rate, selective fertilization will occur. This question can not be decided definitely at present, but two different lines of evidence point toward chance fertilization.

1. Flowers from a single plant pollinated by different males show no decided difference in rate of fertilization.

2. Color differences are transmitted in expected ratios.

Further, it will be recalled that beginning with the F_2 generation, sister plants crossed together have given us our F_3 and F_4 populations, and that these F_3 and F_4 populations apparently have given a constantly increasing percentage of cross-sterility. This is what should be expected under the theory that a small difference in germ plasm constitution is as active as a great difference in causing the active stimulation to pollen tube growth. Breeding sister plants together in succeeding generations causes an automatic increase of homozygosity as is well known. This being a fact, cross-sterility should increase. Such an increase in cross-sterility has been observed in the F_3 and the F_4 generations, but it would not be wise to maintain dogmatically that it is significant.

There are various questions, including the important one of the origin of self-sterility, that can not be discussed at this time. In conclusion, therefore, let us turn once more to the phenomenon of self-sterility in *Ciona intestinalis*. It seems to me that the hypothesis outlined above has few, if any, drawbacks when applied to self-sterility in plants. The question there, as far as we have gone, is one of pollen tube growth, and the theory that the secretion of the direct stimulant can be called forth only by a gamete that differs in its constitution from the somatic

cells between which the pollen tube passes, is logical. If the same theory is to be extended to animals, however, it follows that the external portions of the membranes of the animal egg that have been shown by the wonderful investigations of Loeb and of Lillie to have such important functions, must be functionally zygotic in character. I am aware that this suggestion may be considered pretty radical, but it certainly should be given consideration. I do not like to draw an analogy between the animal egg and a pollen grain, but it may be mentioned that in these structures—surely comparable to the animal egg in the fineness of their membranes and walls—both color and shape are inherited as if they were zygotic in nature.

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THE BLACK-AND-TAN RABBIT AND THE SIGNIFICANCE OF MULTIPLE ALLELOMORPHS

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It is well known that the European rabbit has undergone great variation, and now exists in a large number of domesticated varieties. Darwin and most other naturalists speak of this as "variation under domestication," implying that domestication has caused the variation. Modern genetic research, however, indicates that domestication has occasioned the preservation rather than the origin of the fundamental variations involved. But to what extent man through selection is able to modify the fundamental variations which nature occasionally produces as sports is still an open question. Evidence is nevertheless accumulating that certain of these fundamental variations may occur in two or more alternative forms, and the question then arises (1) whether these alternative forms have arisen independently by distinct acts of mutation, or (2) whether one has arisen from another by a process of secondary mutation, or (3) whether one may not have been transmuted into another by a more or less gradual process. Toward the testing of these several hypotheses much genetic research is now being directed. The first step to be taken is evidently to ascertain in how many alternative forms the same fundamental variation may occur and how these forms are interrelated. A further step will be the attempt to produce new alternative forms at will. It is our purpose, in this paper, to discuss a newly discovered alternative form (allelomorph) of the gray, or agouti, type of coat found in wild rabbits. It occurs in the variety known as black-and-tan.

This variety appears to have arisen from the wild gray, or agouti, type without the loss of any known genetic factor, but by a modification in one. Simple loss of genetic factors is believed by most students of genetics to have

given rise to black, chocolate and albino varieties of rabbits and other rodents, but a hypothesis of this sort will not fit the present case. No factorial loss can be detected, but only a change in that genetic factor which has been called the *agouti* or gray factor. Under the influence of this factor, what would otherwise be a black variety becomes gray, and what would otherwise be sooty yellow ("tortoise" of the fanciers) becomes clear yellow ("fawn" of the fanciers). This same factor converts chocolate into cinnamon (Punnett, 1912). In every way, accordingly, its influence on the coloration of rabbits is similar to that of the agouti factor in guinea-pigs and mice.

But in mice Cuénot (1909) showed that the agouti factor may assume three distinct forms allelomorphic to each other, the effects of which are seen respectively in gray, light-bellied gray, and in yellow mice. Entire absence of agouti marking from the fur (non-agouti) forms a fourth allelomorph in the series.

In guinea-pigs the agouti factor assumes two alternative conditions, the effects of which are seen in ordinary (light-bellied) agoutis and in agoutis with "ticked" bellies, respectively (Detlefsen, 1914). These two conditions correspond closely in appearance and in order of dominance to the light-bellied gray and the ordinary gray of mice, the former being dominant in both cases. Non-agouti is an allelomorph to both, as in mice.

The peculiarity of the agouti seen in black-and-tan rabbits is that it produces less extensive ticking of the fur than does ordinary agouti. In a typical black-and-tan rabbit the light-colored (yellowish) bands on the hairs, which constitute the "ticking," occur only sparingly on the sides of the body, and not at all on the back or the head. But the under side of the body, including the throat and under surface of the tail, are light (yellowish or whitish) and the back of the neck and inside of the ears bear reddish or yellowish pigment, as in gray rabbits. The typical black-and-tan rabbit of the fanciers has very intense pigmentation which deepens the shade of the "tan" (yellow) found on belly, sides, etc. But this inten-

sity is inherited independently of the agouti factor as crosses with dilute colored varieties of rabbit show. For a cross between black-and-tan and blue produces in F_2 (1) *blue-and-tans* as well as (2) black-and-tans, (3) blacks, and (4) blues. This result is strictly parallel with that obtained by crossing intense gray rabbits with blue ones. In that case there are produced (1) blue gray, (2) intense gray, (3) black, and (4) blue young in F_2. It is evident that in each case a dihybrid cross is made and that the end products are the same in the two series except for the difference in the agouti marking of varieties (1) and (2). The natural conclusion is that black-and-tan contains an alternative form of agouti to that found in gray rabbits. If so, it should be capable everywhere of substitution for gray, wherever the latter occurs throughout the entire series of color varieties, and indeed this appears to be the case.

That the black-and-tan factor, like the ordinary agouti factor, is independent of the extension-restriction pair of allelomorphs is shown by a cross of black-and-tan with sooty yellow (*i. e.*, non-agouti yellow or "tortoise"). F_2 contains (1) black-and-tan, (2) black, (3) yellow ("fawn"), and (4) sooty yellow ("tortoise") young. The first two are varieties with extended pigmentation, and the second two are varieties with *restricted* pigmentation; further, varieties (1) and (3) contain *modified* agouti, but varieties (2) and (4) do not.

If a gray rabbit had been used, instead of a black-and-tan, in making the cross just described, three of the four varieties obtained in F_2 would have been indistinguishable from those enumerated, and the fourth one would merely have been gray instead of black-and-tan. This supports the view that black-and-tan is merely an alternative form of gray.

Further, we have evidence to show that the black-and-tan form of agouti, like the agouti of gray rabbits, is independent of the genetic factors which respectively produce Dutch pattern, English pattern, and angora coat, since we have been able to produce individuals in which black-and-

tan was associated with each one of these Mendelizing characters, as well as others in which it was not associated with them. Finally Haecker (1912) has shown that black-and-tan, like the ordinary form of agouti, is independent of albinism, since when black-and-tans are crossed with Himalayan albinos, not only these two varieties are obtained in the F_2 generation, but also *blacks*. The proportions in which these three varieties were obtained by Haecker approximate the modified dihybrid ratio, 9 black-and-tan: 3 black: 4 Himalayan. One of the two Mendelian pairs concerned is color *vs.* albinism; the other and independent one, black-and-tan *vs.* black.

It is known that if a gray rabbit is used, instead of a black-and-tan one, in a cross with Himalayan albinos, the same 9:3:4 ratio is obtained in F_2, of grays, blacks, and albinos, respectively. The observed results differ, in the two cases, only in the substitution of gray for black-and-tan, which is further evidence that it is only another form of the same genetic factor.

Notwithstanding all this consistent and converging evidence, it is possible that the modified form of agouti seen in black-and-tan is not due to a changed agouti factor itself, but to the modifying action of a factor associated with it which partially inhibits its action. Here we must consider two subordinate possibilities: (*a*) that the supposed modifier is wholly independent of the agouti factor, and (*b*) that it is coupled with the agouti factor. The first possibility is readily disproved; the second one is not so easily disposed of.

(*a*) If black-and-tan were due to the action of an independent modifying factor associated with agouti, a cross of black-and-tan with ordinary black should permit the separation of agouti from its supposed modifier in a considerable part of the F_1 gametes and F_2 zygotes, so that we should expect F_2 to contain gray animals as well as blacks and black-and-tans. But experiments started several years ago at the Bussey Institution show that when black is crossed with black-and-tan no gray offspring are obtained either in F_1 or in F_2, but only black-and-tans in

F_1, and black-and-tans and blacks in F_2. This result shows that black-and-tan is a simple dominant over black.

To establish the allelomorphism of black-and-tan with gray the following experiments may be cited. A black-and-tan rabbit heterozygous for black was crossed with a pure-bred Belgian hare, which variety possesses the genetic color factors of wild rabbits, including the ordinary agouti factor. All the F_1 young were gray, closely resembling Belgian hares, but proved to be genetically of two types. For, when mated with black rabbits, some of them produced gray young and black young, while others (even when mated, as in some cases, with the same black animals) produced gray young and black-and-tan young. This result was quite what was to be expected if gray, black-and-tan and black are mutually allelomorphic conditions. On no other hypothesis which we can suggest was it to be expected. For the black-and-tan parent in the cross was known to be heterozygous for black. It accordingly should form two sorts of gametes, black and black-and-tan respectively, provided that these conditions are allelomorphic to each other. The Belgian hare parent was known to transmit gray in all its gametes. The combinations expected from the cross are therefore of two types, viz.: (1) gray combined with black, and (2) gray combined with black-and-tan. It is well known that gray and black are allelomorphs of each other, the former being dominant. Zygotes of type (1), therefore, should produce gametes of two sorts, gray and black; and when back-crossed with black should produce equal numbers of gray young and black ones but no black-and-tan young. We have tested 12 F_1 gray young from this cross (6 males and 6 females) which are evidently of type (1). Mated with black animals, they have produced 69 gray young, and 65 black ones, but no black-and-tans.

On the other hand 8 F_1 gray rabbits from the cross under discussion have proved to be of type (2), producing gray young and black-and-tan young but no black ones. Together they have produced 44 gray and 51 black-and-tan young, besides 14 other young (two litters) which

were certainly not blacks, since they had light bellies, but which died before attaining the age at which gray can be distinguished from black-and-tan. It is certain that among the 109 young produced by the 8 animals of type (2) not a single one was black.

But if black-and-tan is not an actual allelomorph of gray, black young as well as black-and-tans should have been produced in the foregoing case. For if black-and-tan is not allelomorphic with gray, or is due to an independent inhibitor of gray, then an F_1 gray should produce gametes of *four* sorts, rather than as indicated of *two* sorts; *i. e.*, gametes should arise which transmit *both* gray and black-and-tan, and others which transmit *neither* gray nor black-and-tan. The former sort possibly might not be capable of immediate detection in the back-cross with black, but the latter should be readily discovered since they would necessarily produce black young (neither gray nor black-and-tan). The total absence of black young from the litters produced by type (2) matings therefore indicates strongly that gray and black-and-tan are allelomorphs of each other.

(*b*) An alternative view, however, deserves consideration. If gray and black-and-tan are not actual allelomorphs, it is conceivable that they may each be closely "coupled" with a common structure in the germ cells and so *behave* as allelomorphs under ordinary circumstances, though not being such in reality. Or, what would give the same practical result, gray and black-and-tan might be supposed to contain the same agouti factor, but this might be considered in one as closely coupled with a modifying factor which made its action different. Neither form of this hypothesis is capable of proof or disproof, for which reason alone the hypothesis is unimportant, but its probability grows less the larger the number of records obtained which show no breaking of the supposed coupling. Our cases are not as yet numerous enough to throw much light on this question, but so many cases have already been discovered in which characters assume three or more mutually allelomorphic conditions and in which

no evidence of coupled modifiers has yet been discovered, that the existence of such assumed modifiers seems at present doubtful.

Besides the triple or quadruple series of agouti allelomorphs now known for mice, guinea-pigs and rabbits, at least three other Mendelian factors concerned in the pigmentation of rodents vary discontinuously in this way.

1. Castle (1905) and Punnett (1912) have shown that the Himalayan rabbit possesses a form of albinism allelomorphic with that of ordinary albino rabbits, and that both are allelomorphic to ordinary pigmentation. Guinea-pigs show an even more extended series of albino allelomorphs (Castle, 1914, Wright, unpublished data).

2. Punnett (1912) has discovered in rabbits an alternative form of the "extension" factor, one in the presence of which the agouti factor produces a less amount of ticking than normally. He describes it as a *darkened* extension, *i. e.,* as ordinary extension modified by a coupled darkening factor. This is of course only an alternative form of statement to saying that extension occurs in two forms, for he discovered no cases in which the hypothetical coupling was broken. The three allelomorphs in the case of Punnett's rabbits were accordingly: 1, *ordinary extension;* 2, *darkened extension,* and 3, *restriction.*

3. In still another Mendelian factor affecting the pigmentation of rodents discontinuous variation occurs attended almost certainly by the formation of a series of allelomorphs. Cuénot (1904) stated that white-spotting in mice occurs in a graded series of conditions as regards the amount or extent of the white areas. He found that widely separated stages in the series Mendelize on crossing, *i. e.,* that the segregates fluctuate about modal conditions corresponding roughly with the conditions of spotting found in the respective parents crossed, and he concluded the number of allelomorphs which it would be possible to find in the series to be indefinitely great. Subsequent studies of the subject made by Little (1914) in mice, and by Castle and Phillips (1914) in rats, have not served to simplify the matter, and yet they confirm Cué-

not's general idea that a series of mutually allelomorphic conditions of spotting exists. Unquestionably in rats, "hooded" and "Irish" are such modal conditions of spotting, allelomorphic with each other and with the unspotted or self condition (Doncaster, 1905; MacCurdy and Castle, 1907; Castle and Phillips, 1914). The last-named authors find that independent factorial modifiers probably affect the extent of the spotting and yet that, aside from such modifiers, the spotting factor proper may assume relatively stable allelomorphic conditions which Mendelize when crosses are made between stages sufficiently distinct. The point of especial interest in allelomorphic conditions of spotting is that they are *not* perfectly stable, but are capable of gradual and apparently indefinite modification through the selection of fluctuations either plus or minus. It would be premature to conclude that similar fluctuations (though perhaps less conspicuous ones) do not occur about the modal conditions of other genetic factors which show allelomorphic variation. The black-and-tan form of agouti certainly fluctuates in the amount of ticking found on the sides of the body and the head; doubtless some of this fluctuation may be due to factors genetically distinct from the chief allelomorphic factor concerned, but there is at present no sufficient ground for supposing the chief factor itself to be incapable of fluctuation. Indeed, it seems highly probable, in the light of evidence already obtained, that the present modal condition of the black-and-tan character is one which has been attained only as a result of persistent selection, and that reversed selection will carry it back appreciably nearer to the modal condition seen in gray rabbits. Accordingly, it appears doubtful whether allelomorphs are themselves perfectly and permanently stable. Moreover, the rapid increase of recognized allelomorphs makes us wonder whether their number is limited and definite. Black-and-tan represents, on the whole, an intermediate condition between black and gray. Is it not conceivable that intermediates may yet be discovered be-

tween black-and-tan and black, or between black-and-tan and gray, or even that black-and-tan itself might be displaced to such an intermediate condition by selection of its fluctuations? Here are fruitful fields of inquiry to be cultivated before we conclude with the exponents of "exact" heredity that selection of fluctuations is useless and that only mutations count in evolution.

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DATA ON A PECULIAR MENDELIAN RATIO IN DROSOPHILA AMPELOPHILA

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A MUTANT with pink eyes was found by Professor T. H. Morgan in the summer of 1910, in one of his culture bottles which contained wild, red-eyed *Drosophila*. He described it as follows:[2]

"The pink eye is more translucent than the red eye, but of about the same general tone. It lacks the dark fleck seen in the red and vermilion eye when the eye is examined with a lens. This black fleck changes its position as the lens travels over the eye. The pink eye, P, is with a little experience easily distinguished from the other colors, especially in newly hatched flies. When the fly gets old the eye turns to a brown color very characteristic of this type of eye."

Pink was found to be recessive to red. The Mendelian expectation in the F_2, viz., three red to one pink, gave the following (Morgan, 1911):

TABLE I

	F_1	F_2		Proportion of Red : Pink
		Red	Pink	
Red ♀ × Pink ♂..........	All red	3,063	169	18 : 1
Pink ♀ × Red ♂..........	All red	1,133	237	5 : 1

The expectation in either case was 3:1, but the numbers realized were 18:1 and 5:1.

In the spring of 1912 I repeated this experiment under the direction of Professor Morgan in order to find whether or not the above ratio would persist. The results have already been published (Morgan, 1912), but a brief summary is here reproduced for reference:

[1] From the Zoological Laboratory of Columbia University.

[2] *Jour. Exp. Zoology,* Vol. 11, No. 4, November, 1911.

TABLE II

Red ♀ × Pink ♂ → in F_2				Pink ♀ × Red ♂ → in F_2			
Bottle	Red	Pink	Proportion of Red: Pink	Bottle	Red	Pink	Proportion of Red : Pink
A....	318	94	3.3 : 1	*a*....	541	124	4.3 : 1
B.....	140	34	4.1 : 1	*b*....	199	63	3.2 : 1
C.....	375	70	5.3 : 1	*c*.....	582	136	4.3 : 1

The above are records of mass cultures. When pairs were used, the fluctuations in ratio were much more marked. The records of 40 pairs gave an almost unbroken series running from 1.8:1 up to 6:1. In seven cases out of the 40 (18 per cent.) the pink flies exceeded the expectation; 3 pairs (7.5 per cent.) gave a 3:1 ratio, while in the remaining 30 pairs (75 per cent.) the pink fell behind. The total number produced by these 40 pairs was 4,056, of which 891 were pink—an average ratio of 3.58:1, about the same as that shown in Table II.

In a second experiment the F_1 hybrids were back-crossed to the pink. The expectation was 1:1. But the records of 15 bottles of mass culture showed fluctuations running from 1:1 up to 2.3:1. The total number counted in these back crosses was 5,527, of which 2,391 were pink, giving an average ratio of 2.31:1. The pink flies fell behind again, and in about the same proportion as in the normal cross.[3]

These remarkable fluctuations were observed at the time the experiments were in progress, and it was suggested that some environmental condition was responsible for the results by either accelerating or retarding[4] the development of the one or of the other variety. The fact that all these experiments were performed at the same time, and the bottles kept side by side in a room in which a nearly constant temperature was maintained throughout the winter, precludes the chance of a factor outside the culture bottles operating here. Attention was there-

[3] For a detailed account of these experiments see Morgan, 1912.

[4] It should be noted here that owing to the danger of overlapping of generations, the bottles were discarded on the tenth day (counting from the day the first F_2 emerged) regardless of the number of unhatched pupæ

fore directed to the condition of the food inside the bottles. An examination seemed to indicate that those in which the food was dry yielded the higher pink ratios. To test this, two of the bottles in which conditions were normal, and in which the F_2 had just begun to emerge, were made "wet" by the addition of a considerable amount of banana juice. But they still showed a similar tendency to yield a relatively higher proportion of pink.

To ascertain more definitely whether or not moisture or dryness affected in any way the development of these flies, a special experiment was arranged in which some flies were bred in "dry" bottles, and some in "wet" bottles. In the first case, the banana was thoroughly dried by means of filter paper which was discarded after it had absorbed all the available moisture, and the banana wrapped in fresh paper; in the second, banana juice was added every second or third day, so that there was throughout the experiment an abundant amount of wet food in the bottles. The effect of this treatment is shown in Tables III and IV:

TABLE III

Record of F_2 of a Cross Red by Pink in Which the Flies Developed in Bottles in Which the Food was "Dry"

Pink ♀ × Red ♂						Red ♀ × Pink ♂					
Bottle	Red		Pink		Proportion Red : Pink	Bottle	Red		Pink		Proportion Red : Pink
	♀	♂	♀	♂			♀	♂	♀	♂	
A....	95	86	25	18	4.2 : 1	*a*....	91	109	28	32	3.3 : 1
B.....	73	78	20	21	3.7 : 1	*b*....	76	65	29	25	2.6 : 1
C.....	182	164	39	44	4.3 : 1	*c*.....	43	42	14	15	3.0 : 1
D....	99	89	39	42	2.3 : 1	*d*....	161	117	55	46	2.7 : 1

TABLE IV

Record of F_2 of a Cross Red by Pink in Which the Flies Developed in Bottles in Which the Food Was "Wet" from the Beginning

Pink ♀ × Red ♂						Red ♀ × Pink ♂					
Bottle	Red		Pink		Proportion Red : Pink	Bottle	Red		Pink		Proportion Red : Pink
	♀	♂	♀	♂			♀	♂	♀	♂	
A....	91	90	27	38	2.8 : 1	*a*....	82	89	17	17	5.0 : 1
B.....	21	26	12	12	2.0 : 1	*b*....	14	15	8	6	2.0 : 1
C.....	84	83	22	23	3.7 : 1	*c*.....	21	29	2	6	6.0 : 1

TABLE V

RECORD OF 14 ♀'S, 7 RED AND 7 PINK, BRED IN 7 BOTTLES EACH OF WHICH CONTAINED ONE RED AND ONE PINK FERTILIZED FEMALE. THE TABLE SHOWS THEIR RELATIVE PRODUCTIVITY

	Counted on	Bottle *a*				Bottle *b*				Bottle *c*				Bottle *d*				Bottle *e*				Bottle *f*				Bottle *g*			
		Red		Pink		Red		Pink		Red		Pink		Red		Pink		Red		Pink		Red		Pink		Red		Pink	
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Hatched from deposits of April 15–20	Ap. 25	7	4	0	0	3	3	0	0	5	3	0	0	20	12	0	0	15	10	0	0	16	7	3	0	Fertilized by sterile ♂	...	14	0
	26	9	9	0	0	12	8	5	0	7	7	0	0	3	5	0	0	2	6	0	0	18	8	0	6		...	11	9
	27	2	9	8	4	1	8	12	15	7	3	3	1	1	0	1	0	7	12	1	2	4	4	0	0		...	5	8
	29	5	5	9	8	7	5	5	5	20	5	3	2	...	...	...	...	11	15	1	1	7	8	2	5		...	5	6
	30	7	7	7	6	2	5	6	5	12	9	3	2	...	...	...	...	9	10	2	0	14	10	7	6		...	6	3
	May 1	4	4	12	14	3	4	4	8	14	12	3	9	...	...	...	...	9	14	5	0	18	15	8	10		...	5	8
	2	16	15	12	11	13	11	7	12	1	3	1	1	...	...	...	...	11	8	2	1	6	4	6	4		...	14	6
	3	12	27	11	13	20	18	11	7	...	...	...	1	...	...	...	...	20	12	6	6	0	0	1	2		...	4	9
	4	1	2	2	3	5	12	2	4	...	...	...	...	...	...	...	...	31	18	4	1	...	...	...	...		...	17	17
	6	...	...	...	...	2	0	0	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...		...	...	...
Hatched from deposits of April 20–25	Ap. 30	3	3	0	0	15	3	0	0	...	...	...	...	13	1	0	0	4	0	0	0	Killed Ap. 20				0	2	0	0
	May 1	4	2	5	7	15	4	9	3	2	0	0	0	18	7	0	0	6	9	7	12	...	...	...	...	10	9	2	0
	2	0	0	5	5	7	5	2	1	1	4	0	0	8	6	2	0	4	3	6	11	...	...	...	...	4	5	2	4
	3	2	1	4	3	14	11	4	3	2	5	0	0	0	0	0	0	10	8	20	20	...	...	...	...	15	6	9	18
	4	0	2	8	11	8	7	1	2	3	0	1	1	0	0	0	0	1	1	5	7	...	...	...	...	16	13	13	19
	6	died Ap. 23	...	1	6	25	20	2	0	13	6	0	1	0	0	1	0	6	0	8	2	...	...	...	...	20	19	14	20
	8		...	2	0	16	17	4	4	7	5	0	0	0	0	1	0	17	15	15	16	...	...	...	...	4	6	13	7
	9		...	...	...	13	15	6	5	6	7	0	0	...	...	...	...	4	6	13	7	...	...	...	...	5	2	8	4
	10		...	...	...	6	6	4	7	6	2	0	0	...	...	...	...	0	4	0	2	...	...	...	...	2	6	6	4
	11		...	...	...	0	1	2	2	0	1	0	0	...	...	...	...	0	2	0	1	...	...	...	...	...	...	...	...
	Total....	72	90	92	101	187	163	86	85	106	72	14	18	63	31	5	0	167	153	95	89	83	56	27	33	76	68	148	142
																						Deduct bottle 1....				...	...	81	66
																										76	68	67	76

It is evident that neither dryness nor moisture has any effect in rendering more favorable the conditions needed for the emergence of either the red or the pink variety. The results show that under unfavorable conditions, large numbers of larvæ and pupæ fail to develop, since the total yield of each and every bottle is far below the normal output; and those which do emerge are but chance survivals.

It was suggested that the reason the pink flies fell behind the expected ratio, was the fact that the mutant was weaker than the wild stock and therefore less likely to come through the larval and pupal stages. If this were the case, they should always fall behind. In many cases, however, they actually exceeded the expectation. Furthermore, they always seemed to be just as vigorous, and to live as long as the wild fly.

The hypothesis which was formed at this stage, and which determined to a large extent the experiments which followed was, that another factor not related to eye-color was at work. Such a factor, if one is assumed to be present, by its independent action might be responsible for the disturbance in ratio. It might, moreover, be present in the wild stock which originally gave rise to the pink, since the wild fly is similarly, though less frequently, affected. It is with the search for such factors that the subsequent experiments will chiefly deal.

Before presenting the data, it will be well to point out some of the possible sources of error which were to a great extent eliminated.

I. The method usually employed in these experiments is as follows: The flies, which are to be cross-bred, are taken out of the culture bottles as soon as they hatch and before they have time to mate. They are then put into a clean, sterilized bottle in mass cultures of about five or more pairs. There they remain till their offspring (F_1) are ready to emerge: 9–10 days in summer and 11–12 or 13 days in winter when the temperature is low. The F_1 flies are placed in fresh bottles for a similar length of

time, and then removed. During the succeeding ten days the F_2 are counted each day as they hatch. The bottle is then discarded for fear of overlapping of generations; for, the F_2 might mate and deposit eggs before removal.

It has, however, been observed that each time a bottle is discarded a considerable number of pupæ and even larvæ remain behind. This being the case, it is possible that the ratio we get does not always represent what actually happens. In order to count the total output, it was decided to transfer the flies to a second bottle on the fifth day. All eggs deposited, during the five days that the parent remained there, would thus have at least fifteen days to develop. It was hoped that, in this way, a more representative ratio would be obtained.

II. It was shown (Tables III and IV) that large numbers of larvæ fail to develop when a bottle becomes too "dry" or too "wet." Considerable care was taken to avoid either of these conditions. If a bottle showed a tendency to dry up, fresh food was immediately added; when it was too wet, the moisture was absorbed by filter paper.

III. The yield of a mass-culture bottle is always relatively small as compared with that of the same number of flies mated in pairs. This would indicate probably greater mortality due to overcrowding. For this reason only pairs were used in the later experiments.

In the first of these experiments pure stocks, both pink and red, were used; for it was believed that if differences existed other than the red-pink distribution, between the two varieties, they would be more emphasized if hybridization had not been effected. The chief purpose, however, was to become familiar with the modes of behavior of the races. The experiment follows:

A number of flies, both pink and red, were isolated within one to six hours after hatching and the sexes kept apart for 3–4 days, after which time they were mated, red to red, pink to pink. Immediately after mating, which took place within five minutes to two hours, the males

were removed. One red and one pink of these females were put into each of seven bottles. In this way the same environmental conditions were secured for the eggs of both. Five days later they were allowed to mate again (not by the same males) and placed in a second set of bottles. The same two females that were together in the first set were also together in the second. There they remained five more days. Counts of the flies that hatched were made from day to day, and the bottles were emptied as long as they continued to yield. The results are given in Table V.

The records of these flies show several interesting and suggestive facts. It will be noted, in the first place, that the length of larval life varies through wide limits. Each bottle contained eggs which were deposited during a period of no more than five days. The hatching periods, however, extended through eleven days in the first set of bottles (April 25–May 6) and twelve days in the second set (April 30–May 12). The flies which emerged first consumed but ten days for development; those which emerged last took at least sixteen days. This phenomenon was more marked where the number produced was larger, suggesting that crowding may retard the development of some individuals.

Of equal interest is the fact that the pink flies invariably began to hatch from 24 to 48 hours later than the red. This was true in the second set of bottles as well as in the first, which proves that it was not due to late maturity of the parents, for, at the time of transfer, they were in the midst of their productive period.

Another point of interest is to be found in the fact that the pink stock was, on the whole, less fertile than the red. In the two bottles '*a*' and '*g*' where the productivity of the two was about equal the red, like the pink, were also low-producers. This is significant, and will be referred to later.

In order to test the above-mentioned facts, the following experiment was performed. F_1 hybrids were mated

in pairs, and transferred during a period of twenty days (May 16–June 4) 13 times, remaining in each bottle from one to two days. Care was taken to count every fly of the F_2 that hatched. The result follows:

TABLE VI

Record of F_2 Flies Which Hatched from Eggs Deposited During a Period of 20 Days; During Which Time the Parents Were Transferred Thirteen Times

Pink ♀ × Red ♂ → in F_2						Red ♀ × Pink ♂ → in F_2					
Pair	Red		Pink		Proportion Red : Pink	Pair	Red		Pink		Proportion Red : Pink
	♀	♂	♀	♂			♀	♂	♀	♂	
A....	235	209	90	95	2.3 : 1	*a*....	134	118	42	37	3.2 : 1
B.....	220	213	84	75	2.7 : 1	*b*....	105	108	67	64	1.6 : 1
C.....	162	168	65	58	2.7 : 1	*c*.....	189	153	52	53	3.2 : 1
D....	202	119	14	19	9.7 : 1	*d*[5]....	70	58	11	8	6.5 : 1

Here we have a group in which the pink ran sometimes relatively ahead of the red. But the other extreme is also represented in pairs *D* and *d*. The numbers obtained are in each case large enough to be significant.

This experiment was repeated on a larger scale in the fall of 1912. Ten pairs were used for each of these crosses and they were continually transferred as long as they lived. The records follow:

TABLE VII

F_2 Record from Red-eyed Flies Crossed to Pink-eyed Flies Mated in Pairs. Showing the Total Output of Each F_1 Pair During its Lifetime

Pink ♀ × Red ♂ → in F_2

Pair	No. of Days Each Pair Lived[6]	No. of Times Transferred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each Pair	Proportion Red : Pink
			♀	♂	♀	♂				
A ..	45	27	604	578	89	81	1,282	170	1,452	7.5 : 1
B ..	19	15	169	145	30	41	314	71	385	4.4 : 1
C...	31	22	320	305	116	94	625	210	835	3.0 : 1
D ..	63	32	658	681	203	221	1,339	424	1,763	3.2 : 1
E ..	20	16	218	218	79	73	436	152	588	2.9 : 1
F...	47	28	476	435	153	144	911	297	1,208	3.0 : 1
G...	22	17	259	248	83	89	507	172	679	2.9+ : 1
H ..	32	22	392	396	152	125	788	277	1,065	2.8+ : 1
I...	63	32	864	805	258	281	1,669	539	2,208	3.1 : 1
J...	25	19	357	358	130	124	715	254	969	2.8 : 1

[5] Lived only eight days.

[6] The length of time a fly lived should not be taken as a criterion for measuring its vigor. In most cases death is accidental.

TABLE VIII

F_2 Record from Red-eyed Flies Crossed to Pink-eyed Flies Mated in Pairs. Showing the Total Output of Each F_1 Pair During its Lifetime

Red ♀ × Pink ♂ → in F_2

Pair	No. of Days Each Lived	No. of Times Transferred	Red		Pink		Total Red	Total Pink	Total No. Produced	Proportion Red : Pink
			♀	♂	♀	♂				
a...	28	20	366	294	124	112	660	236	896	2.8 : 1
b...	13	11	163	170	58	49	333	107	440	3.1 : 1
c...	7	5	74	90	22	28	164	50	214	3.2 : 1
d...	14	12	213	195	58	57	408	115	523	3.5+ : 1
e...	22	15	101	106	33	26	207	59	266	3.6 : 1
f...	21	16	215	266	95	78	481	173	654	2.8 : 1
g...	37	24	358	288	113	139	646	252	898	2.6 : 1
h...	45	27	433	444	179	169	877	348	1,225	2.2+ : 1
i...	43	26	546	533	187	185	1,079	372	1,451	2.9 : 1
j...	30	21	298	306	90	83	604	173	777	3.5 : 1

A comparison of Tables VI, VII and VIII suggests the possible presence of high and low pink-producing "strains" in these stocks. To test this, some of the offspring of pair *A* (Table VII) in which the ratio was 7.5 of red to 1 of pink, were inbred for the F_3 in order to see if the same ratio would persist. As there were among the red both homozygous and heterozygous forms, they were each mated to their pink sisters or brothers. This combination would give with the former all red (since red is dominant) and with the latter a ratio of 1:1.

Pair *F* (Table VII) in which the ratio was ideal, 3:1, was chosen for the control, and treated in like manner.

In this as in the preceding experiment the flies were mated in pairs and transferred to fresh bottles every second or third day. A peculiar thing happened. Out of 25 pairs taken from "*A*" only two gave offspring; the remaining 23 pairs were apparently sterile. It could not have been due to bad banana or any other unfavorable condition, for the flies had already been transferred five times and no pupæ were found in any of the other bottles. Furthermore, the 16 pairs of the control which ran parallel to them, and were fed with the same food, did well.

To find out whether these flies were actually sterile, each of the 14 remaining pairs—9 having meanwhile died—were separated and every individual mated to wild red-eyed stock. The sterility of the pink flies, both male and female, was found to be absolute, while all red of both sexes were fertile.

As an additional test, some of the offspring of the last cross were inbred *en masse* in order to extract the pink flies which they would produce, since some of them were heterozygous for eye color.

A small number of pink flies were obtained and mated to their red brother and sisters: each pink female was put in a bottle with 3 or 4 red males, and each pink male with 3 or 4 red females. Out of 19 individuals thus tested, only three were found to be fertile; the remaining 16 were sterile.

These facts seem to indicate that some factor or group of factors which make for sterility were present in the "pink"-containing gamete. The results are the more significant since the hybrid fly, in which this condition prevailed, produced a very low pink ratio. Of the control in which 10 pairs were found to be heterozygous for eye-color with an expectation of 1:1, the following results were obtained:

TABLE IX

RECORD OF THE OFFSPRING OF F_2 FLIES OF A CROSS OF PINK ♀ BY RED ♂ IN WHICH A RATIO WAS 3:1, AS EXPECTED. (See pair *F* of Table VIII)

Red Heterozygous ♀ by (Brother) Pink ♂

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	50	352	348	236	244	700	480	1,180	1.7 : 1
2	27	150	130	170	161	280	231	511	1.2 : 1
3	18	51	85	59	50	136	109	245	1.2+ : 1
4	29	251	245	240	258	496	498	994	1.0 : 1
5	10	147	132	133	112	279	245	524	1.1 : 1

The reciprocal cross gave the following:

Pink Heterozygous ♀ by (Brother) Red ♂

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
I	20	82	89	69	59	171	128	299	1.3+ : 1
II	22	123	124	94	107	247	201	448	1.2 : 1
III	32	223	240	213	247	463	460	920	1.0 : 1
IV	29	215	176	184	163	391	347	738	1.1 : 1
V	40	216	178	101	136	394	237	631	1.7 : 1

It will be seen that all the red flies of the last cross were heterozygous, and should give, on further inbreeding, a ratio of 3:1. Four pairs out of five (one being sterile), taken from Pair III of Table IX, gave the following:

TABLE X

Red ♀ × Red ♂ of Pair III, Table IX

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
a....	40	312	348	43	45	660	88	748	7.5 : 1
b....	23	282	316	80	103	598	183	781	3.3 : 1
c....	40	194	214	60	70	408	130	538	3.1 : 1
d....	14	175	163	47	59	338	106	444	3.2 : 1

Pair "*a*" above gave the same result as pair "*A*" (Table VII)—the ratio in each case being 7.5:1. The offspring of the latter were found to contain a high percentage of sterile pink flies, owing to which the attempt then made to test that ratio failed. It was therefore decided to repeat the same experiment with the offspring of this "*a*" pair. As in the former case, the heterozygous red flies were picked out by crossing them to their pink brothers and sisters. The expectation was again 1:1. The records follow.

TABLE XI

Record of Eight Pairs, Heterozygous Red × to Pink, Taken from the Offspring of Pair "*a*," Table X

Pink ♀ × Heterozygous Red ♂

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
A...	27	179	176	79	88	355	167	522	2.1+ : 1
B...	12	155	153	83	66	308	149	457	2.0+ : 1
C...	28	163	205	126	159	368	285	653	1.3 : 1
D...	28	243	222	206	219	465	425	890	1.1 : 1

The reciprocal cross gave:

Red Heterozygous ♀ × Pink ♂

Pair	No. of Days Each Lived	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
a....	28	282	236	210	260	518	470	988	1.1 : 1
b....	28	296	290	5	5	586	10	596	59.0 : 1
c....	12	144	158	136	152	302	288	590	1.0+ : 1
d....	25	72	113	63	98	185	161	346	1.1+ : 1

The pair with which we started (*F*, Table VII) gave the ideal 3:1 ratio; but in each of the three generations which were bred from its offspring (Tables IX, X, XI) there appeared again the same fluctuations which were observed in the preceding experiments, and with even more striking emphasis. Among the offspring of the same pair are found some that give a 3:1 ratio and some that give a 7.5:1; in a second pair we have some giving 1:1, and one giving 59:1. The latter especially suggests the presence of a factor that actually inhibits the development of the pink flies, and, moreover, that it is being segregated in a mixed stock.

If the presence of such factor is assumed, we should be able by inbreeding to select stocks in which it is present and in which it is absent. For this reason the experiment recorded above (Table V) was here repeated with some modifications. It will be remembered that in the former, a fertilized red female and a fertilized pink male were placed in each bottle and their offspring, the F_1, counted. In the following experiment, in order to secure segregation, the F_2 were counted. Four virgin pairs, two red and two pink, were taken out of the culture bottles and mated separately. Of the F_1 of each of the four, six pairs were taken out, 24 pairs in all, and bred for the F_2. Unlike the first experiment, the males in this case were allowed to remain with the females throughout the experiment. This insured sufficient sperms for the eggs. Every second day the food was removed, together with the

eggs deposited upon it, and fresh banana supplied. Each two batches of eggs—one deposited by a red female, one by a pink female—were placed together in one bottle so that they might develop side by side and under the same environmental conditions. The result of this experiment is shown in Table XII.

TABLE XII

F_2 of Two Pink Pairs *A*, *B*, and Two Red Pairs *a*, *b*, Showing Segregation of Productivity. Eggs of *A*I–*a*I, *B*I–*b*I, etc., Were Developed in the Same Bottle

	Pink							Red					
	F_1 Pair	No. of Days Each Lived	♀	♂	Total Produced	Ave. Per Day		F_1 Pair	No. of Days Each Lived	♀	♂	Total Produced	Ave. Per Day
Pair *A*	*A* I	44	450	446	896	20.40	Pair *a*	*a* I	20	233	219	452	22.60
	A II	24	169	224	393	16.36		*a* II	22	9	8	17	0.77
	A III	28	61	67	128	4.57		*a* III	25	63	64	127	5.10
	A IV	30	221	233	454	15.13		*a* IV	31	272	232	504	16.26
	A V	36	331	345	676	18.77		*a* V	30	179	171	350	11.66
	A VI	21	37	33	70	3.33		*a* VI	22	152	138	390	17.73
Pair *B*	*B* I	29	199	217	416	14.34	Pair *b*	*b* I	44	122	104	226	5.10
	B II	24	93	93	186	7.75		*b* II	28	103	77	180	6.43
	B III	35	31	47	78	2.23		*b* III	44	302	256	558	12.70
	B IV	30	15	13	28	0.93		*b* IV	35	22	34	56	1.60
	B V	28	11	10	21	0.75		*b* V	31	302	293	595	19.20
	B VI	18	10	8	18	1.00		*b* VI	44	154	139	293	6.65

Segregation with respect to productivity is here evident. Whether the low fertility[7] seen in so large a proportion of these flies was due to an actually low egg-production, or whether it was due to something which prohibited development or to some defect in the germ cell owing to which fertilization could not be effected, is not known. That one of the latter possibilities is likely to be realized here can be inferred from the work of Dr. R. R. Hyde in this laboratory. He counted the eggs of hundreds of individuals, and compared them with the number of flies which emerged from them. According to

[7] The term "fertility" is used here, as defined by Hyde, to indicate the number of eggs that complete development and give rise to mature flies. (See Hyde, *Jour. Exp. Zool.*, August, 1914, p. 185.)

his observations, only about 75 per cent. of the eggs of the wild fly ever reach maturity, and in some of the mutations no more than 25 per cent. of the eggs develop.

Another point of interest brought out in the last experiment is the fact that the wild, red-eyed fly behaves in exactly the same manner as the mutant pink fly. This may be the reason for the observed shifting of the ratio sometimes in favor of the one variety, sometimes in favor of the other. It shows furthermore that it was not the pink as such that caused the disturbance. The red also might be similarly disturbed and perhaps by the same agent or by another agent that affected the productivity in the same way.

If the abnormally low number produced by some of the pairs of Table XII be due to the inability of a large number of their eggs to develop, and if we assume this character to be transmissible, it must reappear in the F_2 of a cross in which one of the parents possessed this factor, *i. e.*, a large number of individuals, one quarter of the output, should fail to develop. This would be in accordance with Mendelian principles. A number of crosses were therefore made in various combinations with the individuals taken from Table XII. The results follow:

TABLE XIII

F_2 of 16 Pairs of a Cross Red by Pink in Which the Parents Came from *A*I, *a*I, Table XII, the Average Daily Productivity of Which Was 22 and 20, Respectively

A. Pink ♀ (Productivity 20 Per Day) × Red ♂ (Prod. 22 Per Day)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	17	301	294	110	104	595	214	809	2.80 : 1
2	32	484	457	160	147	941	307	1,248	3.01 : 1
3	32	504	570	181	189	1,074	370	1,444	2.90 : 1
4	32	559	548	205	200	1,107	405	1,512	2.73 : 1
5	8	125	126	40	35	251	75	326	3.35 : 1
6	18	261	243	98	89	504	187	691	2.70 : 1
7	21	391	449	155	142	840	297	1,137	2.86 : 1
8	32	673	700	203	236	1,373	439	1,812	3.10 : 1
Total number produced by 8 pairs						6,685	2,294	8,979	

Average proportion, 2.91 : 1

B. Red ♀ (Productivity 22 Per Day) × Pink ♂ (Prod. 20 Per Day)

Pair	No. of Days Each was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
I	30	415	395	135	127	810	252	1,062	3.21 : 1
II	14	132	121	41	73	253	114	367	2.22 : 1
III	24	317	337	97	94	654	191	845	3.42 : 1
IV	30	484	378	119	120	862	239	1,101	3.60 : 1
V	24	392	418	96	140	810	236	1,046	3.42 : 1
VI	30	656	592	198	200	1,248	398	1,646	3.14 : 1
VII	30	566	647	182	209	1,213	391	1,504	3.10 : 1
VIII	24	438	451	132	153	889	285	1,174	3.10 : 1
Total produced by 8 pairs.................						6,739	2,106	8,845	

Average proportion, 3.24 : 1

If the proportion of red to pink, realized in the F_2, depends upon the relative fertility of the two parents which form the cross, we should get in this case, where the parents were supposedly equally fertile, the ideal 3:1 ratio. The records, however, show considerable fluctuations. Nevertheless, these results are perfectly in accord with our hypothesis. Looking back to Table XII, which furnished the parents of this cross, the explanation is obvious. The averages per day for *A*I–*A*VI were 20, 16, 4, 15, 18 and 3, respectively. Similarly, *a*I–*a*VI gave 22, 0.7, 5, 10, 16, 11 and 17, respectively. It is therefore reasonable to assume that among the offspring of *A*I (productivity 20) and *a*I (productivity 22) individuals should be found which would repeat the series. Fluctuation is, therefore, to be expected. The average of many such pairs, however, should be 3:1. The proportion obtained was 2.91:1 in one case; 3.24:1 in the other, or a general average of 3.08:1.

It should also be noted here that in this as well as in the subsequent experiments, wherever eight pairs are recorded, they are not the offspring of one, but of two distinct crossings of one pair each which were made at the same time; that pairs 1–4, 5–8; I–IV, V–VIII, respectively, were brothers and sisters. More than one line is thus represented in each case. With these facts in mind, we may pass on to the remaining experiments.

TABLE XIV

F_1 of 12 Pairs of a Cross Red by Pink in Which the Parents Were *A*I and *b*II (Table XII) the Average Daily Productivity of Which Was 20 and 6, Respectively

A. Pink ♀ (Productivity 20 Per Day) × Red ♂ (Productivity 6)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	32	355	331	117	95	686	212	898	3.23 : 1
2	28	340	373	126	132	713	258	971	2.76 : 1
3	19	330	288	120	124	618	244	862	2.52 : 1
4	32	544	562	188	188	1,106	376	1,482	2.94 : 1
5	23	574	578	203	214	1,152	417	1,569	2.76 : 1
6	14	196	194	66	59	390	125	515	3.12 : 1
7	32	648	603	222	246	1,251	468	1,719	2.67 : 1
8	32	429	374	141	144	803	285	1,088	2.81 : 1
Total number produced by 8 pairs.........						6,719	2,385	9,104	

Average proportion, 2.84 : 1

B. Red ♀ (Productivity 6 Per Day) × Pink ♂ (Productivity 20)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
I	28	488	482	176	177	970	353	1,323	2.74 : 1
II	6	83	78	21	30	161	51	212	3.15 : 1
III	28	463	465	164	153	928	317	1,245	2.93 : 1
IV	21	375	383	114	116	758	230	988	3.20 : 1
Total number produced by 4 years.......						2,817	951	3,768	

Average proportion, 2.96 : 1

In most of these pairs the pink slightly exceeded the 3:1 expectation. In the few in which they fell behind, the red (if we assume fertility to be the cause) might have been of a higher fertility than the pink, as has been explained. As a group, however, they give a proportion somewhat below 3:1.

In the next cross, the red fly was the more fertile. The results are given in the following table:

TABLE XV

F₂ OF A CROSS RED BY PINK IN WHICH THE PARENTS WERE *BIV* AND *aI* (TABLE XII) THE AVERAGE DAILY PRODUCTIVITY OF WHICH WAS 1 AND 22, RESPECTIVELY

A. Pink ♀ (Productivity 1 Per Day) × Red ♂ (Productivity 22)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	22	398	405	120	105	803	225	1,028	3.52 : 1
2	28	512	477	107	125	989	232	1,221	4.26 : 1
3	22	420	389	104	135	809	239	1,048	3.34 : 1
4	18	340	300	86	106	640	192	832	3.33 : 1
5	19	420	428	129	134	852	263	1,115	3.24 : 1
6	19	372	396	100	104	768	204	972	3.76 : 1
7	17	198	220	58	83	418	141	559	3.00 : 1
8	17	174	212	62	62	386	124	510	3.10 : 1
Total number produced by 8 pairs						5,665	1,620	7,285	

Average proportion, 3.50 : 1

B. Red ♀ (Productivity 22 Per Day) × Pink ♂ (Productivity 1)

Pair	No. of Days Each Was Bred	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
I	31	264	262	101	87	526	188	714	2.80 : 1
II	27	353	350	80	85	703	165	868	4.26 : 1
III	31	430	403	97	112	833	209	1,042	4.00 : 1
IV	31	427	420	111	128	847	239	1,086	3.54 : 1
V	28	486	471	122	147	957	269	1,226	3.60 : 1
VI	28	573	568	139	154	1,141	293	1,434	3.90 : 1
VII	28	505	505	116	127	1,010	243	1,253	4.15 : 1
VIII	24	502	501	134	140	1,003	274	1,277	3.66 : 1
Total produced by 8 pairs						7,020	1,880	8,900	

Average proportion, 3.73 : 1

Of the 16 pairs of this cross only one gave less than 3 : 1. In the remaining 15, the proportion was, in each case, considerably higher than 3 : 1. It will be noted that of all 16 pairs that one was the least fertile. This would indicate, on the hypothesis suggested, that the gamete containing the "red" factor did not have relatively as high a potential of fertility as did the parent which produced it.

A comparison of Tables XIV and XV shows that we have two distinct groups: one in which the extracted pink exceed the expectation, and one in which they fall behind

the expectation. Yet the method employed in each case was the same; the history of each is the same. The only difference is to be found in the fact that in the one case the pink came from a more fertile parent; in the other, the red.

The offspring of pairs "7" (Table XIV) and "2" (Table XV) in which the ratios were 2.67:1 and 4.26:1, respectively, were inbred for the F_3. Fifteen pairs were taken from each, but as there were among the red both homozygous and heterozygous flies, only eight gave pink in each case. The results follow:

TABLE XVI

Record of 8 Pairs Heterozygous Red-eyed F_2 of Pair "7" (Table XIV) in Which the Ratio Was 2.67:1

Pair	No. of Days	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	14	270	279	80	78	549	158	707	3.47 : 1
2	14	159	167	58	42	326	100	426	3.26 : 1
3	14	5	7	2	1	12	3	15	4.00 : 1
4	14	135	143	46	49	278	95	373	2.93 : 1
5	14	183	157	49	50	340	99	439	3.23 : 1
6	14	172	154	45	42	326	87	413	3.74 : 1
7	14	142	137	43	49	279	92	371	3.00 : 1
8	14	57	46	12	16	103	28	131	3.67 : 1

TABLE XVII

Record of 8 Pairs Heterozygous Red-eyed F_2 of Pair "2" (Table XV) in Which the Ratio of Red to Pink Was 4.26:1

Pair	No. of Days	Red		Pink		Total Red	Total Pink	Total No. Produced by Each	Proportion Red : Pink
		♀	♂	♀	♂				
1	14	295	308	100	97	603	197	800	3.06 : 1
2	14	392	326	97	105	718	202	920	3.55 : 1
3	14	281	288	95	86	569	181	750	3.14 : 1
4	14	110	104	32	33	214	65	279	3.31 : 1
5	14	167	170	70	59	337	129	466	2.61 : 1
6	14	314	277	82	86	591	168	759	3.52 : 1
7	14	160	169	32	41	329	73	402	4.50 : 1
8	14	133	146	49	41	279	90	369	3.10 : 1

These results are significant, in that they show that the original ratios, which their parents gave, were lost.

The fact that it has been found possible by proper manipulation to get a group in which the ratio fluctuated in one direction only, even if it was not as marked as was hoped it would be, indicates that the disturbance is due to an *internal,* and not to an external, cause. This was further emphasized by the distinct tendency for segregation, as was to be expected if there were some heterozygous individuals. It was also suggested in another way. No matter how short-lived or how long-lived a pair was; whether it was transferred once, twice, or even twenty times, the ratio of red to pink did not vary throughout its life when the yields of the several bottles were compared with one another.

TABLE XVIII

F_2 OF FOUR PAIRS (*A, B, C* AND *D*) RED ♀ × PINK ♂ IN WHICH THE MALES WERE CROSSED EACH TO SEVERAL OF HIS OWN DAUGHTERS

Expectation 1 : 1

Pink ♂	No. of ♀	Total Red	Total Pink	Proportion Red : Pink
A ×	1	97	45	2.15 : 1
	2	105	71	1.46 : 1
	3	231	161	1.43 : 1
	4	171	122	1.40 : 1
	5	211	194	1.10 : 1
B ×	1	110	80	1.38 : 1
	2	263	251	1.00 : 1
	3	242	198	1.28 : 1
	4	183	143	1.28 : 1
	5	231	189	1.22 : 1
	6	162	102	1.62 : 1
C ×	1	201	113	1.78 : 1
	2	257	116	2.22 : 1
	3	255	206	1.27 : 1
	4	206	153	1.36 : 1
	5	243	197	1.23 : 1
	6	125	98	1.27 : 1
D ×	1	179	135	1.30 : 1
	2	217	121	1.80 : 1
	3	107	74	1.44 : 1

TABLE XIX

F_2 OF PAIRS *A, B, C* AND *D* OF TABLE XVIII

Expectation 3 : 1

F_1 of Pair	No. of Pair	Total Red	Total Pink	Proportion Red : Pink
A	1	153	41	3.73 : 1
	2	158	55	3.00 : 1
	3	96	21	4.60 : 1
B	1	140	43	3.27 : 1
	2	215	72	3.00 : 1
	3	136	71	1.92 : 1
C	1	50	21	2.38 : 1
	2	125	43	2.90 : 1
	3	122	49	2.49 : 1
	4	193	67	2.88 : 1
D	1	26	6	4.33 : 1
	2	112	34	3.30 : 1
	3	194	74	2.62 : 1

The proportion of red to pink was found to bear a direct relation to the relative "fertility" of the parents which produced the hybrid. This suggests a causal relation between the two.

In dealing with "fertility" the difficulty that one encounters is, that the offspring of any pair may, with respect to this character, differ from either parent, and also differ amongst themselves, forming a graded series running from the most to the least fertile. An individual taken from such a population is an indefinite quantity and will often defeat the purpose of the experiment. In order to simplify this as far as possible, the following experiment was planned:

Four red-eyed, virgin females were each mated to a pink male. Each male was again crossed to several of his own daughters. The records are given in Table XVIII. As a control a number of F_1 pairs were bred in each case. The records are given in Table XIX.

A graphic representation of all pairs recorded in Tables VI–XIX, except for the several very unusual ratios, is given in Figs. 1 and 2.

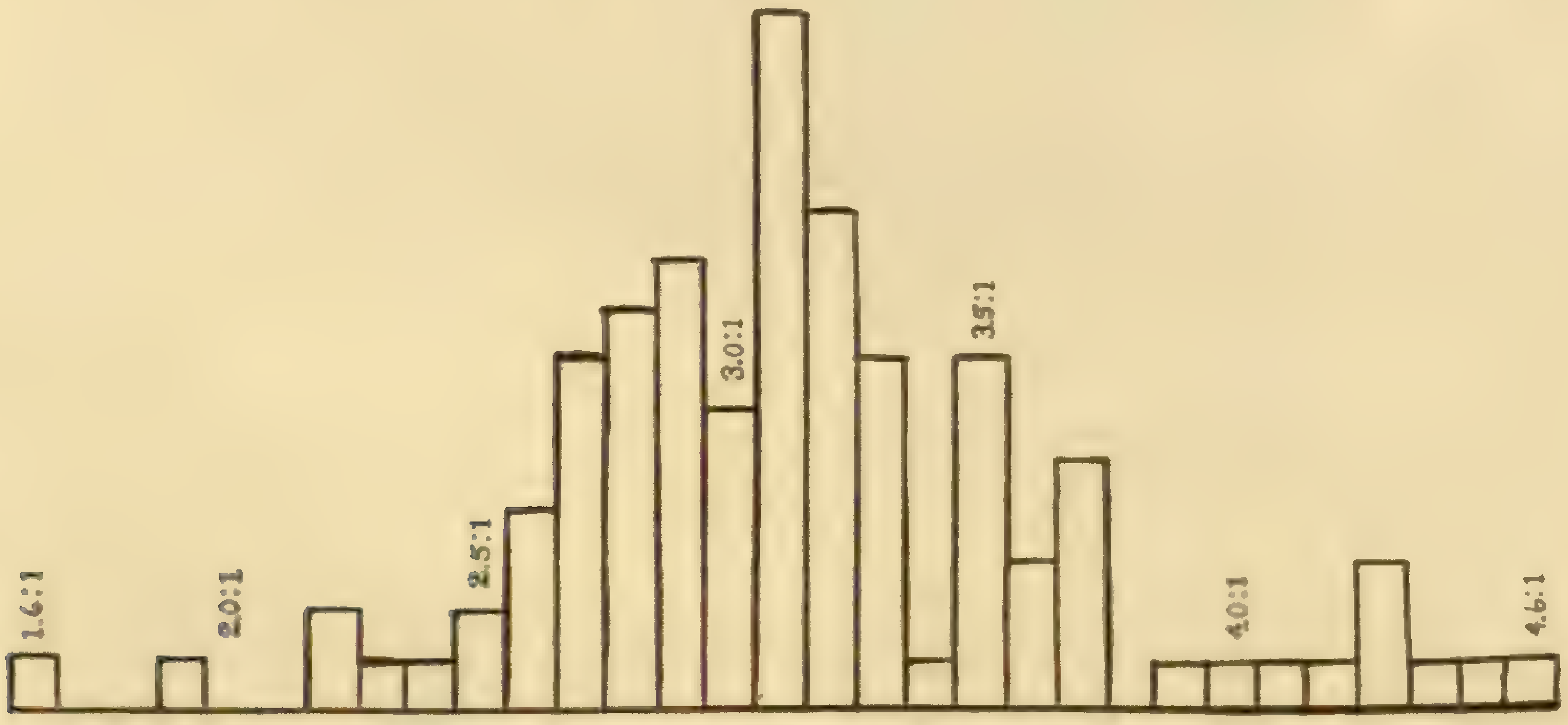

Fig. 1

Fig. 1 contains 99 pairs in each of which the expected ratio was 3:1, with a total population of 82,607. They are distributed as follows:

No. of Pairs		Ratios of Red : Pink
1	gave	1.6 : 1
0	gave	1.7 : 1
0	gave	1.8 : 1
1	gave	1.9 : 1
0	gave	2.0 : 1
0	gave	2.1 : 1
2	gave	2.2 : 1
1	gave	2.3 : 1
1	gave	2.4 : 1
2	gave	2.5 : 1
4	gave	2.6 : 1
7	gave	2.7 : 1
8	gave	2.8 : 1
9	gave	2.9 : 1
6	gave	3.0 : 1
14	gave	3.1 : 1
10	gave	3.2 : 1
7	gave	3.3 : 1
1	gave	3.4 : 1
7	gave	3.5 : 1
3	gave	3.6 : 1
5	gave	3.7 : 1
0	gave	3.8 : 1
1	gave	3.9 : 1
1	gave	4.0 : 1
1	gave	4.1 : 1
1	gave	4.2 : 1
3	gave	4.3 : 1
1	gave	4.4 : 1
1	gave	4.5 : 1
1	gave	4.6 : 1
Total . . 99		

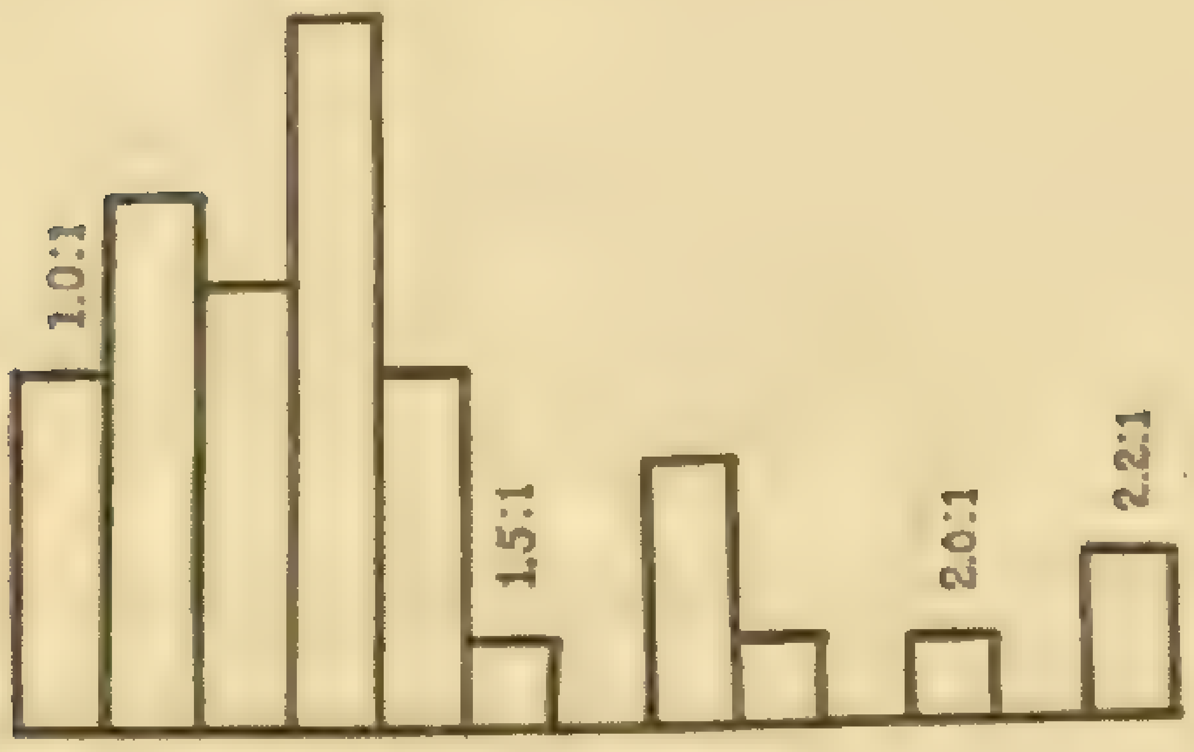

Fig. 2

Fig. 2 contains 37 pairs of back-crosses; expected ratio 1 : 1; total population 17,008. They are distributed as follows:

No. of Pairs		Ratios of Red : Pink[8]
4	gave	1.0 : 1
6	gave	1.1 : 1
5	gave	1.2 : 1
8	gave	1.3 : 1
4	gave	1.4 : 1
1	gave	1.5 : 1
1	gave	1.6 : 1
3	gave	1.7 : 1
1	gave	1.8 : 1
0	gave	1.9 : 1
1	gave	2.0 : 1
1	gave	2.1 : 1
2	gave	2.2 : 1
Total. .37		

To these should be added:

1 pair which gave 9.7 : 1, Table V
1 pair which gave 6.5 : 1, Table VI
1 pair which gave 7.5 : 1, Table VII
1 pair which gave 7.5 : 1, Table X
1 pair which gave 59.0 : 1, Table XI

Except for the several detached pairs at the extreme limits, Fig. 1 shows a normal curve. A disturbance of 0.5 in either direction (less than 10 per cent.) is quite within the limits of experimental accuracy. The larger disturbances, ratios of 6 or 7 : 1, and also the first results reported by Morgan ('11 and '12) are yet to be explained. These are too large to be attributed to experimental error.

The data presented in the foregoing pages show that there has been a marked improvement in the ratio of pink to red since 1911. In one case only (1913) was the disturbance greater than those of Morgan (59 : 1). The remaining very marked disturbances were between 6 and 10 : 1. And these appeared so infrequently that in mass-cultures their presence would hardly have been felt.

A corresponding improvement has also been observed in the fertility of the pink-eyed race between 1912 and 1913. This is seen on comparing Tables V and XII. In the first, the fertility of the pink was much lower than that of the red; in the second (about one year later), it was as high.

Hyde ('14) showed that in some races of *Drosophila*

[8] None gave a ratio of less than one red to one pink.

ampelophila, the number of eggs failing to reach maturity is between 25 per cent. and 75 per cent. of the total output; and concluded that this peculiarity probably behaves as a Mendelian recessive factor. More recently, Morgan ('14) describes recessive lethal factors in *Drosophila,* which he defines, "as any factor that brings about the death of the individual in which it occurs, provided that its effect is not counteracted by the action of its normal allelomorph."

In the light of this evidence, the following conclusions suggest themselves:

1. The original pink-eye mutant was heterozygous for some non-sex-linked factor which, in the homozygous state, acts like Morgan's lethal. This factor was, in the course of time, to a large extent eliminated, as is to be expected if the individuals homozygous for it are more likely to die. The chance of such homozygous forms appearing again, has thereby been much reduced. This is borne out by, and also explains, the improvement in the pink race.

2. A similar recessive, though not necessarily the same factor, might also be present in some individuals of the wild, red-eyed stock. Hyde's work mentioned above gives weight to this assumption—which is not at all an unreasonable assumption in a species as unstable as this. judging by the vast number of mutations reported. For this reason, the red sometimes fall behind the expected ratio.

3. The mode of action of these lethals shows that they are linked to the "pink" factor or to its normal "red" allelomorph. This will be clear from the following analysis:

Of the flies recorded in Fig. 2, one parent was *RP* (with gametes *R* and *P*); the other was *PP* (with gametes *P* and *P*). The zygotes resulting from these gametes almost invariably give fewer *PP*'s than *RP*'s. In other words, the homozygous forms run behind the heterozygous forms. The relation between these two classes may also be supposed to hold in the F_2 cross (Fig. 1). Here, however, the reds (*RR* and *RP*) run relatively less often ahead of

PP. This must be due to a deficiency in the homozygous *RR* flies. In other words, the results taken all together (Figs. 1 and 2) show that the disturbance is brought about by factors (in the third chromosome) which in the homozygous state act as lethals or perhaps as semi-lethals. Random introduction of one or two or no lethals may be assumed, as follows:

(*A*) If the lethal is introduced by the "pink-bearing" chromosome, the homozygous pink will be depressed in the F_2.

(*B*) If introduced by the "red-bearing" chromosome, the homozygous red will be depressed in the F_2.

(*C*) If two lethals, both of which are identical, are introduced at the same time, one by the red and one by the pink, all classes will be equally depressed,[9] and the results as far as concerns the F_2 ratio will be the same as if there were no lethals present, *i. e.*, the 3:1 ratio will be realized.

(*D*) If two lethals that are different are introduced at the same time, one by the red and one by the pink, both the homozygous classes (*RR* and *PP*) will be depressed, but not the *RP*. There would be somewhat fewer pinks than expected in the F_2.

I wish to acknowledge my indebtedness to Professor T. H. Morgan, whose kind attention and suggestions both throughout the foregoing experiments and in the preparation of the present report, were invaluable. I also wish to express my appreciation to Mr. H. J. Muller to whom I owe some suggestions concerning the interpretation of the results.

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[9] "Crossing-over" is ignored as the character of the results is not changed thereby.

SHORTER ARTICLES AND DISCUSSION

SELECTION, SUGAR-BEETS AND THRIPS

A DISCOVERY of great importance to students of genetics has recently been made by one of the plant-breeders[1] of the U. S. Department of Agriculture, viz., that beets are regularly cross-pollinated and that an important agent in the process is a minute inconspicuous insect, so small that it readily passes "through the meshes of fine silk chiffon."

To understand fully the theoretical importance of this discovery one need only recall the large attention given to the sugar-beet in recent adverse criticisms of the selection-theory. De Vries in his "Mutationstheorie," p. 72, cites the case of the sugar-beet as showing the most systematic, refined and elaborate selection known for any cultivated plant, and yet as being without any permanent effect in raising the sugar content of the beet. For, although the average sugar content of the beet has by systematic selection been practically doubled in the last 60 years, De Vries holds the improved racial condition to be unstable and thinks that the improved race would within a few generations revert to its old level of sugar-content if the selection were discontinued. His reason for thinking so is the familiar fact that the offspring of the *best* selected beets are on the average not quite so good as their selected mother-beets, but show a tendency to regress downward toward the old level of sugar content. It should be pointed out, however, that in reality regression is not toward the *original* average of 7 or 8 per cent. sugar-content, but toward an average twice as high as this. For De Vries's variation polygon (*l. c.*, Fig. 22) for the sugar content of 40,000 beets shows a nearly symmetrical probability curve about a mode at 15.5 per cent. It is to be supposed therefore that regression would occur *toward this* condition from both the upper and the lower halves of the frequency polygon, rather than toward the old average condition of 7–8 per cent., which, according to the data of DeVries, is now rarely if ever seen in the improved race. To have doubled the average sugar-content of the beet is certainly something of an achievement for selection; the form of

[1] Shaw, Harry B., "Thrips as Pollinators of Beet Flowers," Bull. No. 104, U. S. Dept. Agr., July 10, 1914.

the variation polygon indicates that the change is permanent, so far as ordinary racial characters have permanency.

But why, it may be asked, has selection not achieved *more* in this case? Why should the descendants of, say, a 25 per cent. beet not score better than this? There are probably several reasons why. (1) Physiological reasons probably offer obstacles. A beet can not be formed which is *all* sugar. There has to be present in the beet a machinery for *manufacturing* the sugar. Perhaps 25 per cent. is an impossibly high average for a race of beets. (2) Perhaps the exceptional 25-per-cent. beet owes its extra sweetness in part to environmental causes which are not permanent. In that case the extra sweetness is "somatic rather than germinal," as we should say in the case of an animal.

(3) Finally the discovery that beets are never self-fertilized, but in every generation are cross fertilized, explains why improvement of the beet through selection is so slow and tedious a process. What progress could the animal breeder expect to make if he were able to select only the dams, but never the sires, for his flocks? This is the condition which confronts the plant breeder in attempting to improve the sugar beet. The animal breeder is often chided with the small numbers which his experiments yield as compared with the enormous numbers which an experiment with plants may produce, but the animal breeder has at least this satisfaction that when the animals are securely penned there need be no uncertainty about pedigrees.

The careful observations of Shaw show that thrips, so common in the blossoms of plants and yet so minute as easily to escape notice and to penetrate within silk nets and under paper bags, may be a cause of unsuspected cross-pollination and unaccountable "mutation" in the breeding of cereals and other plants.

W. E. Castle

Bussey Institution,
October 24, 1914

A NOTE ON MULTIPLE ALLELOMORPHS IN MICE

Professor T. H. Morgan has recently published in this journal the results of some of his experiments on color inheritance in mice. In this paper he offers material which he considers "evidence establishing" a series of multiple allelomorphs. His series consist at present of four forms, "yellow, gray white-belly, gray

gray-belly and black." The essential point of his conclusion is that no more than two of these conditions can be transmitted by any one animal.

The fact that Cuénot in his series of classic papers on color inheritance in mice (1902–1911) recognizes these same four types as forming a group of allelomorphs is not mentioned by Morgan, whose paper, without knowledge of Cuénot's work, might well be taken to contain "the evidence establishing this series of allelomorphs" as he himself considers that it does. Since Morgan appears to have overlooked Cuénot's work with these forms, it may be interesting to give a brief statement of Cuénot's results.

As early as 1903 Cuénot recognized that albinos, potentially yellows, when crossed with black gave besides yellow offspring *either* black or agouti young, but not both. This is, of course, evidence that yellow, agouti and black are all allelomorphic to one another. In 1904 he gives formulæ (p. 46) showing that he considers this to be the case. At the same time he gives the ratios produced by crossing an albino potentially a *heterozygous gray* (agouti) with a yellow carrying black, but no agouti, and albinism. For present purposes the albinism in the cross is negligible. Cuénot recognized that the ratio expected from this cross was 2 yellow, 1 black and 1 agouti (gray). He obtained 34 yellow, 20 black and 16 agouti; the calculated numbers being 38 : 19 : 19. Sturtevant (1912) in discussing the allelomorphism or coupling of black, agouti and yellow in mice has also overlooked Cuénot's results, for in mentioning the cross of a heterozygous agouti with a yellow carrying black, he states "apparently Morgan is the only one who has reported such a cross. He obtained 4 yellows, 5 agoutis and 1 black."

To return to Cuénot's work; in 1907 he made a report on the hereditary behavior of the white bellied agouti variety (gris à ventre blanc) which he considers allelomorphic to yellow, agouti and black. On page 10 in speaking of *determinants* he says: "Il y en a le même nombre dans les races unicolores et dans la race grise; ces races diffèrent, non pas par la quantité de leurs déterminants mais par la qualité." This is essentially the idea underlying multiple allelomorphism. Later in the same paper he says of G, the agouti determinant ". . . il présent un grand nombre de mutations: G′, N et J." (G′ = white bellied gray; N = black and J = yellow.) On page 13 he tabulates the varieties, *in order of their dominance,* yellow, white-bellied agouti,

agouti and black. Morgan reached the same order of dominance in 1911 and has recently (1914) recorded them, beginning with black, as follows:

b = black,
B^G = gray gray-belly,
B^w = gray white-belly,
B^y = yellow.

In 1908 Morgan published certain facts concerning the inheritance of the white-bellied gray pattern. Cuénot at once (1908) publicly called Morgan's attention to the similarity of their material and added facts which showed that he had already investigated the inheritance of this same pattern in 1907. Morgan later acknowledged its similarity.

In 1911 Cuénot states plainly (p. 47): "Les souris jaunes sont characterisées par un déterminant J, allélomorphe a G, G′ et N, at qui les domine tous dans les croisements . . . il n'y à que les zygotes renfermant J dominant un autre déterminant allélomorphe (G, G′ ou N) qui peuvent évolouer."

Morgan's 1914 paper adds several detailed matings and records the testing of yellows of both sexes. However, in most respects, his work corroborates the pioneer experiments of Cuénot and does so in such detail that he falls into the same error as did Cuénot in considering "black" as a necessary member of the allelomorphic series. This is obviously incorrect for the whole series of allelomorphs exists equally well in forms *utterly lacking the ability to produce black pigment* as some of Morgan's experiments showed. The true series of allelomorphs is yellow, white bellied agouti, gray-bellied agouti and *non agouti* (not black).

C. C. Little

Bussey Institution,
October 19, 1914

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ON THE TIME OF SEGREGATION OF GENETIC FACTORS IN PLANTS

IN *Œnothera lamarckiana,* Geerts (3) has observed that two microspores of each tetrad abort. From the results of reciprocal crosses, De Vries (9) concluded that there was a segregation of genetic factors between the aborted and unaborted pollen-grains. In my crosses of *Stizolobium* species (2), half of the pollen-grains abort in a random manner in the anthers of the F_1 hybrids; and I can only explain the results of the breeding work on the hypothesis that there is a segregation between the four microspores of each tetrad. Hence I conclude that the segregation does not take place before the cell-divisions which form the pollen-mother-cells, but takes place in the divisions which form the microspores. In other words, segregation occurs here, not among the cells of the diploid generation, but at the moment of formation of the individuals of the haploid generation.

In the ovules of *Stizolobium* crosses I have shown that there is a random segregation of aborted and normal embryo-sacs; and this agrees with the observations of Geerts on the functional megaspores of *O. lamarckiana*. If somatic segregation occurred, there would be a segregation of whole ovaries or parts of ovaries with ovules all aborted or all normal, causing a distribution which would differ markedly from the binomial distribution demanded by a random segregation according to the law of chance. I have shown that with lots of n ovules each, the distribution of the aborted and normal ovules corresponds to the binomial $(1+1)^n$. Hence segregation can not have taken place before the formation of the nucellus of the ovule.

In many species and varieties of *Citrus,* as Strasburger (7)

and Osawa (4) have proved (and as I can confirm), embryos are formed from the tissue of the nucellus adjacent to the embryo-sac. I have also shown (1), as Strasburger suspected, that a similar mode of formation prevails in certain varieties of *Mangifera indica.* In F_1 hybrids between certain *Citrus* species (8), these adventive embryos do not show segregation; and the adventive embryos of a mango variety give plants nearly constant to that variety. Hence segregation had not taken place when the cells surrounding the megaspore-mother-cell were formed.[1] The same conclusion follows on the work of Ostenfeld (5) and Rosenberg (6) with certain *Hieracia.*

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[1] Somatic segregation is not the only available hypothesis in the cases of the double Matthiola and Petunia. For the double stock *may* have one half or less of its pollen-grains ineffective for fertilization (compare Correns on the pollination of *Mirabilis jalapa,* in *Ber. Deutsch. Bot. Ges.*, Bd. 18, S. 422–435); and in the petunia doubleness *may* be incompletely dominant, as in the greenhouse carnation.

NOTES AND LITERATURE

REPULSION IN WHEAT[1]

THE evidence was furnished by the (F_2) of the cross: Smooth Black × Rough White. "Smooth Black" is a wheat obtained from the (F_2) of the Rivet × Fife cross and it breeds quite true. Its glumes are absolutely glabrous and of a burnished black color. "Rough White" is the well-known Essex Rough Chaff Wheat. The glumes are very hairy and of the ordinary white color. The (F_2) sorted into the following classes:

Rough Black	Rough White	Smooth Black	Smooth White
120	43	47	3

The expectation for the 1 : 3 : 3 : 1 repulsion is:

109.8	49.9	49.3	3.3

"Blackness" is probably not a simple character for in the (F_2) various degrees of it occur—the patches of it on the glumes being of various sizes and intensities of color. There is evidence that it is closely connected with the "gray" color of Rivet glumes.

F. L. ENGLEDOW

THE DETERMINATION OF THE BEST VALUE OF THE COUPLING-RATIO FROM A GIVEN SET OF DATA[1]

MR. G. N. COLLINS has suggested in this journal[2] a general method for determining the value to assign to the coupling-ratio for a given set of data. He has worked out the value of a coefficient of association for the whole series of possible integral ratios 1 : 1 : 1 : 1, 2 : 1 : 1 : 2, etc., and then used the observed value of the same coefficient to decide which ratio gives the best agreement with the facts. The method is very simple, but does not lead to the value which is the most advantageous in a certain sense. If F_1, F_2, F_3, F_4, are the set of theoretical frequencies for a given value of the ratio and if F_1', F_2', F_3', F_4', are the observed fre-

[1] "A Case of Repulsion in Wheat," by F. L. Engledow, St. John's College, Cambridge (Proc. Camb. Phil. Soc., Vol. 18).

[1] F. L. Engledow and G. Udny Yule (Proc. Cambridge Phil. Soc., XVII, 436).

quencies, and if $\chi^2 = \Sigma(F' - F)^2/F$, then the probability p that in random sampling deviations of equal or greater improbability will arise is a function of χ^2 which decreases continually as χ^2 increases. The best value of the ratio will then be that value which makes p a maximum or χ^2 a minimum. The problem taken in the note is to determine this value. Unfortunately the solution is not a simple one, depending on an equation of the fourth degree. A few cases are, however, taken as illustrations and the question of probable error is discussed. The recognized fact that, especially when the coupling-ratio is high, its value may receive considerable alteration without greatly altering the closeness of agreement between theory and fact, receives additional emphasis from some of the results given and makes it clear that considerable caution must be used before attaching importance to the precise values of high ratios.

F. L. E AND G. U. Y.

[2] AM. NAT., XLVI.

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THE

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MUTATION *EN MASSE*

HARLEY HARRIS BARTLETT[1]

During the writer's experiments with *Œnothera* two different species have been discovered of which certain strains give rise by mutation to large numbers of dwarfs. In both cases the dwarfs occur in far greater numbers than experience would lead one to expect, even in the most actively mutant strain. Similar, although not exactly parallel, phenomena have been observed by both de Vries and Davis in certain hybrid *Œnotheras*, but not, as far as the writer knows, in any unhybridized species. Since the cultures have now been continued long enough so that there can be no doubt as to the accuracy of the observations, the least complicated of the two cases is here placed on record. It concerns *Œnothera Reynoldsii* sp. nov. (A technical diagnosis of this species will be published elsewhere.) The seeds from which the cultivated strain arose were collected at Knoxville, Tennessee, in the fall of 1910, by Dr. E. S. Reynolds, then connected with the botanical department of the University of Tennessee.

Fig. 1 is a diagram showing the size and relationship of the cultures of *Œnothera Reynoldsii* which have thus far been grown. No diversity was found in the small F_1 and F_2 cultures, of only ten and five plants, respectively, which were grown in 1911 and 1912. The F_3 generation of twenty-six plants, grown in 1913, exhibited

[1] From the Bureau of Plant Industry, U. S. Department of Agriculture, Office of Plant Physiological and Fermentation Investigations. Published by the permission of the Secretary of Agriculture.

a most unexpected segregation into three marked types, forma *typica,* reproducing the parental form, and two dwarf types, mut. *semialta* and mut. *debilis,* so named because of their resemblance to the two classes of dwarfs which de Vries[2] obtained from *Œ. nanella* × *Œ. biennis.* Mut. *semialta* is about half as tall as f. *typica* and has a

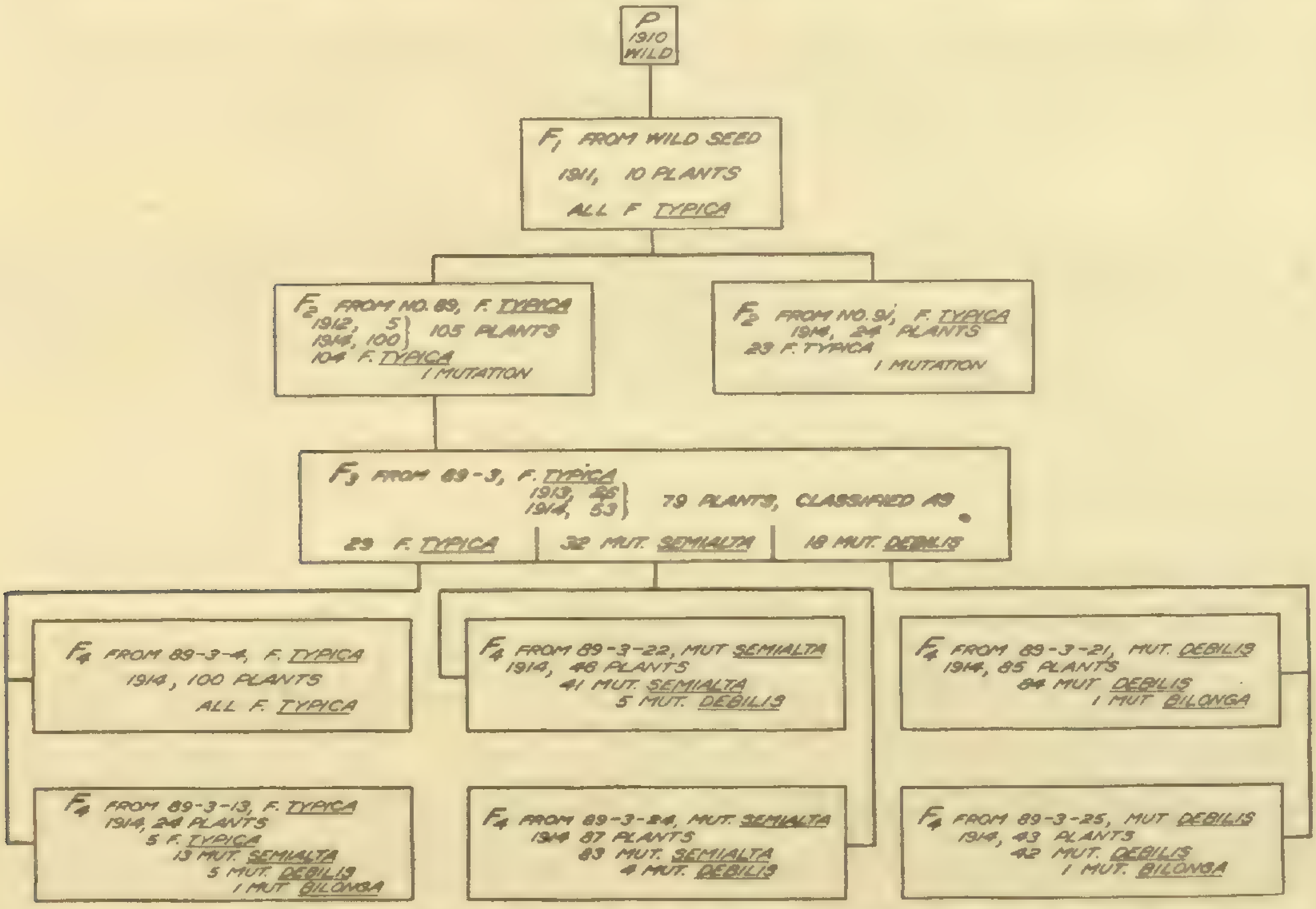

FIG. 1. Diagram showing the size and relationship of the cultures of *Œnothera Reynoldsii,* 1911–1914.

very dense and showy inflorescence in which the fruits and flowers are very little smaller than in the parent form. The leaves, however, are decidedly reduced. Mut. *debilis* is more variable in size than mut. *semialta,* but averages about half as high as the latter. Its fruits and flowers are somewhat reduced, but by no means proportionately to the size of the plant. The leaves, on the contrary, are much more reduced than those of mut. *semialta.* The inflorescence is not as dense, but often longer.

The unlooked-for occurrence of these types in the F_3 of 1913 led to the duplication in 1914 of both the F_2 and F_3 generations from seeds which had been left over from former years. In 100 additional F_2 plants of the mutant

[2] "Gruppenweise Artbildung," pp. 241–244.

strain there were 99 plants of f. *typica* and one mutation of a quite different type from either mut. *semialta* or mut. *debilis*. The original F_3 culture had consisted of 26 plants, including two of f. *typica*, 16 of mut. *semialta*

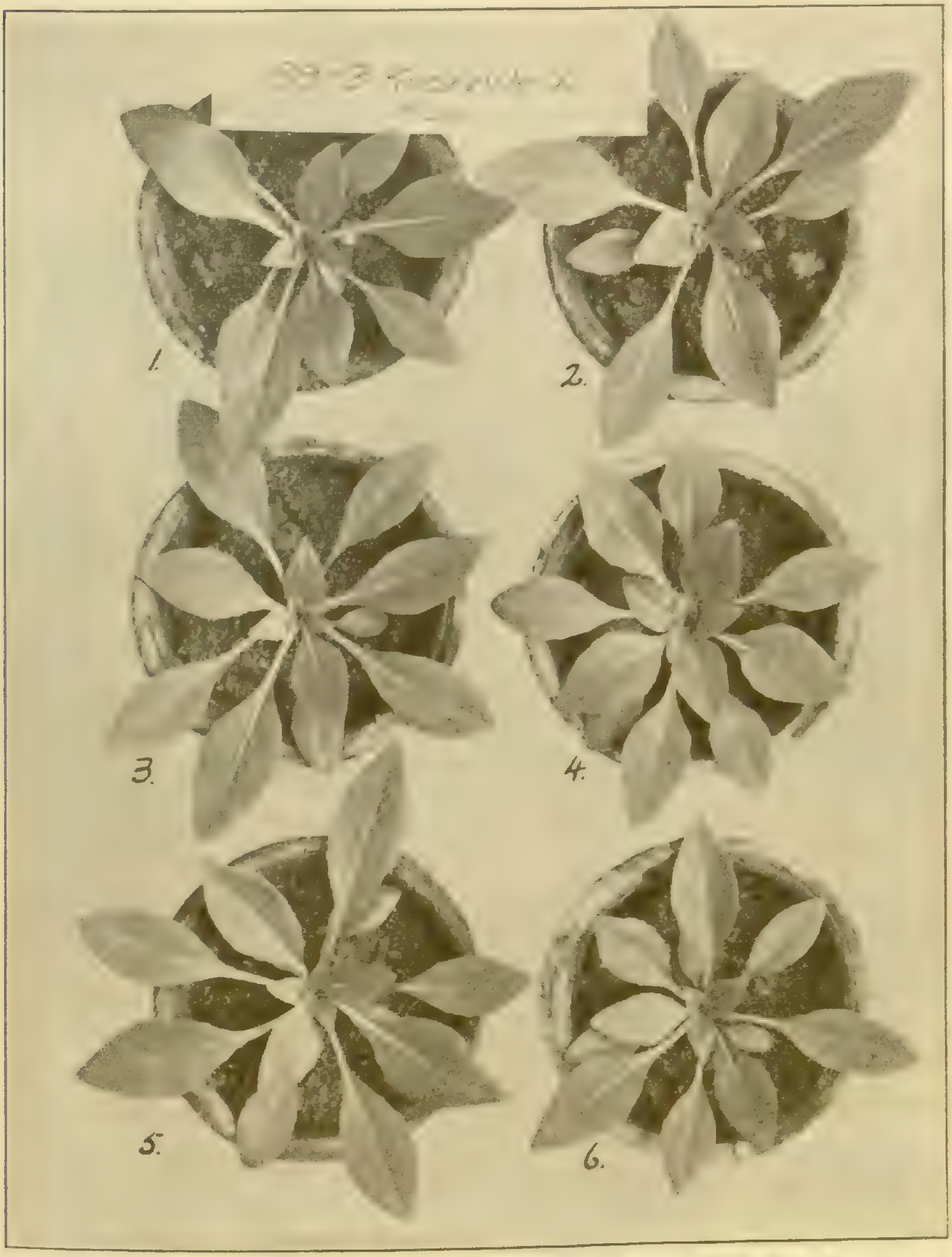

FIG. 2. A random sample of six plants from the F_3 culture of 1913. No. 4 is f. *typica*; the rest are all mut. *semialta*.

and eight of mut. *debilis*. In the supplementary culture of 53 sister-plants, grown in 1914, there were 27 plants of f. *typica*, 16 of mut. *semialta* and ten of mut. *debilis*.

FIG. 3. Adjacent plants of mut. *debilis* (on the left No. 89-3-25, chosen as parent of one of the F_4 progenies) and mut. *semialta* (on the right; No. 89-3-24, chosen as parent of one of the F_4 progenies). The small labels on the plants are 10 cm. long. (Reduction same as in Fig. 4.)

There can therefore be no doubt that the F_2 was an essentially uniform generation and that the F_3 was the first generation to throw the two dwarf types, except perhaps as rare mutations, which were not detected on account of the small size of the cultures. In this connection it may be remarked that the mutations of *Œnothera Reynoldsii* can not be detected in very young cultures with any degree of precision. Up to the time the rosettes are set out in the garden, after they have been started in the greenhouse in pots, they show no consistent differences among themselves. It happens that six seedlings of the 1913 F_3 were photographed before any diversity whatever had been detected in the culture. They must therefore be considered a random sample from the 26 plants. All turned out to be mut. *semialta* except one, which was f. *typica*. The photograph is reproduced as Fig. 2.

At maturity the contrast between the classes is very

striking, and leaves no room for doubt as to the proper classification of any individual. Fig. 3 shows adjacent plants of mut. *debilis* and mut. *semialta;* Fig. 4 mut. *debilis* and f. *typica*. Figs. 5, 6 and 7 show branches of the three forms, on the same scale of reduction.

The F_4 generation, grown in 1914, consisted of the progenies of two plants of each of the three types. The two externally identical parent plants of f. *typica* (there

FIG. 4. Adjacent plants of mut. *debilis* (in front) and f. *typica* (behind; No. 89-3-13, chosen as parent of one of the F_4 progenies). The small labels on the plants are 10 cm. long. (Reduction same as in Fig. 3.)

were only two in the F_3 of 1913) proved to be of very different genetic constitution. The progeny of one, numbering 100 plants, were all strictly like the parent, showing not the slightest deviation from f. *typica*. The other progeny, however, repeated the diversity of the F_3 generation, containing five plants of f. *typica*, 13 of mut. *semialta* and five of mut. *debilis* in a culture of 24 plants. This progeny, also, included one plant of a third dwarf

FIG. 5. *Œnothera Reynoldsii* f. *typica*. Branch from F_3 plant No. 89-3-13, chosen as parent of one of the F_4 cultures. The entire plant is shown in Fig. 4.

mutation, which will be referred to below as mut. *bilonga*.

The two F_3 plants of mut. *semialta* which were used as parents gave very similar progenies, consisting of mut. *semialta* and mut. *debilis*. In one case the numbers were 41 of mut. *semialta* and five of mut. *debilis* in a total of 46; in the other case, 83 of *semialta* and four of mut. *debilis* in a total of 87.

The two progenies from mut. *debilis* parents, containing 85 and 43 plants, respectively, were all mut. *debilis* like the parents, except that each progeny contained one individual of mut. *bilonga*. Before discussing the latter mutation it may be well to capitulate.

1. The individuals of f. *typica* are of two kinds, (*a*) those which do not throw dwarfs, and (*b*) those which throw from 60 per cent. to 80 per cent. of dwarfs.

FIG. 6. Mut. *semialta*. Branches of F_3 plant No. 89-3-23.

2. The dwarfs are of two kinds, one of which, mut. *semialta*, is intermediate between f. *typica* and the extreme dwarf, mut. *debilis*.

3. Mut. *semialta* reproduces itself in the greater part of its progeny, but throws a small number (seemingly about 7 per cent.) of mut. *debilis*.

4. Mut. *debilis* does not throw either f. *typica* or f. *semialta*. It comes true, except for the fact that it rarely throws mut. *bilonga*.

Mut. *bilonga* is by far the most interesting of the variants of *Œnothera Reynoldsii*. It has occurred once as a

primary mutation from f. *typica,* and twice as a secondary mutation from mut. *debilis.* Although mut. *debilis* seems to be an extreme recessive, derived from f. *typica* either by the simultaneous or by the successive loss of two factors for height, it throws mut. *bilonga,* which shows a return to the stature of mut. *semialta.* In fact, mut. *bilonga* would be identical with mut. *semialta* if it were not for the difference in the length of the fruits. It has already been stated that in both mut. *semialta* and mut. *debilis* the fruits are by no means as reduced in size as the foliage and stems. It seems almost as though the

FIG. 7. Mut. *debilis.* Branches of F_3 plant No. 89-3-12. The entire plant is shown in Fig. 4.

process of mutation, which results in the formation of either of these dwarfs, does not involve the factors determining fruit size. In other words, the slight reduction in size seems not to be due to a modification of the hereditary qualities of the plant, but rather to diminished nutrition. If this explanation is the true one, the fruits of mut.

FIG. 8. Branches of mut. *semialta* (left; No. 89-3-13-1) and mut. *bilonga* (right; No. 89-3-13-12) showing the similarity of the foliage and dissimilarity of the fruits, which are twice as long in the latter as in the former. The two mutations were sister-plants in the progeny of an F_3 f. *typica*; No. 89-3-13, shown in Figs. 4 and 5.

debilis are small for the same reason that the late autumn fruits on weak lateral branches of f. *typica* are small. When, by mutation to mut. *bilonga*, mut. *debilis* reassumes the stature and foliage size of mut. *semialta*, there is a modification of the characters which determine the length of the fruit. Not only is the stature doubled, and the length of the leaves doubled, but the length of the fruit is also doubled. Mut. *bilonga* is to all outward appearance the same as mut. *semialta*, except that the fruits are twice as long. Thus, we have the anomalous situation that mut. *bilonga*, a dwarf type, is characterized by the

FIG. 9. Branches of mut. *debilis* (left; No. 89-3-21-1) and mut. *bilonga* (right; No. 89-3-21-85) showing dissimilarity in both foliage and fruits. The two forms were sisters in the progeny of one of the original examples of mut. *debilis* which appeared in the F_3 of 1913.

longest fruits in the subgenus *Onagra*. Exceptional fruits are 70 mm. long; the average length of six hand-pollinated fruits was 62 mm. By way of comparison, it may be stated that the length of the average fruit of f. *typica* is about 33 mm., and that the longest is 38 mm. None of the immediate allies of *Œnothera biennis* have longer fruits than those of *O. Reynoldsii* f. *typica*, although there are allies of *O. muricata* in which the fruits average as long or longer. There is no species, however, in which the fruit length of mut. *bilonga* is even approached. Here we have an apparent case of progressive mutation, which will be tested out as soon as possible. Mut. *bilonga* has not thus far been carried into a second generation. Both it and the two other dwarfs are completely self-fertile,

and furnish an abundance of good seed. It is planned to make a biometrical study of fruit length next year, when the second generation of mut. *bilonga* will be available.

In Fig. 8 the two branches on the left are mut. *semialta;* the two on the right mut. *bilonga.* The plants which furnished the material belonged to an F_4 culture from f. *typica,* containing five plants of f. *typica,* 13 of mut. *semialta,* five of mut. *debilis* and one of mut. *bilonga.* In Fig. 9, on the contrary, the contrast is between sister-plants of mut. *debilis* and mut. *bilonga* in the progeny of mut. *debilis.* A comparison of the figures will show the identity of mut. *bilonga* from the two sources.

The phenomenon presented by *Œnothera Reynoldsii,* called mutation *en masse* for want of a better name, seems of sufficient interest to justify this preliminary paper. The fact that it appears in one of the short-styled, self-pollinating species makes it of especial interest. An explanation can hardly be attempted until the interrelationships of the various derivations have been worked out by a series of crosses. Nevertheless, it seems clear that mutation *en masse* bears a certain degree of resemblance to Mendelian segregation. The fundamental mutation which causes the diversity possibly occurs in only one of the two gametes in a generation preceding the one in which diversity becomes manifest. It is masked by the dominance of the parental characters transmitted through the other gamete. Segregation then occurs in the following generation. No explanation suggests itself for the enormous surplus of dwarfs in the progenies exhibiting diversity, unless perhaps it is that the results are complicated by selective germination or selective mortality. At any rate, the ratios thus far obtained do not conform to any Mendelian expectation. Larger cultures, to be grown next year, may prove more enlightening.

To the mutationist, the most interesting problem presented by *Œnothera Reynoldsii* is the origin of mut. *bilonga* from mut. *debilis,* involving, as now seems probable, the origin of a new character.

THE ALBINO SERIES OF ALLELOMORPHS IN GUINEA-PIGS

SEWALL WRIGHT

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Albinism is one of the most familiar color conditions found in mammals. In all cases it has proved to be a simple Mendelian recessive to the pigmented condition. This was demonstrated for guinea-pigs by Castle and Allen in 1903. In the present paper evidence will be presented showing that in guinea-pigs there are two grades of pigmentation, intermediate between full intensity and albinism, which form with these a series of four allelomorphs with dominance in the order of increasing pigmentation.

The most highly pigmented condition is also the most familiar. To this condition, which we may call intensity, belong those types of guinea-pigs which show in the fur intense black pigmentation or the intense orange-yellow known as "red," or both. Examples are the blacks, the reds, the golden agoutis and the black-and-red tortoise-shells of the fanciers. All of these have black eyes.

The second condition in intensity and order of dominance contains color varieties which have long been known. In these black is reduced to a sepia-brown color much like human brown hair, known very inappropriately as "blue." Red is reduced to yellow or cream. The eye color remains black. Thus we have blues, creams, silver agoutis and blue-and-cream tortoise-shells in place of the four types mentioned as of the intense condition. That these four so-called dilute types, as well as others not mentioned, differ from the intense types by the same factor or factors has long been known. It is shown by the fact that one can start with any of these and by crosses with the appropriate intense variety produce any other

dilute variety in the second generation at latest. The writer has done this for all of the types mentioned by starting with the cream variety.

The third condition is not so familiar as the others. It has appeared at the Bussey Institution only in the descendants of three guinea-pigs brought from Peru by Professor Castle in 1911. Among these black is reduced to sepia (or "blue"), indistinguishable from the "blue" of the dilutes. Red is reduced to white. Not a trace of yellow pigment has been found in guinea-pigs with this allelomorph. One of the most striking features of this condition is the glowing red color of the eyes, easily distinguishable from the black eyes of the intense and dilute guinea-pigs as well as from the pink eyes of the albinos. There is a deficiency of pigment in both retina and iris. Because of this feature this condition will be known as red-eye. It was announced as an allelomorph of albinism by Castle (1914). Permission has very kindly been given the writer to present in this paper data on the red-eye condition, in the work on which he has been associated. With the red-eye factor, the blacks, reds, golden agoutis and black-and-red tortoise-shells become red-eyed blues, red-eyed whites, red-eyed silver agoutis and red-eyed blue-and-white tortoise-shells, respectively. These four varieties have all been obtained by crossing one of them, the red-eyed silver agouti, with various stock guinea-pigs and extracting the different combinations in F_2. The red-eyed white is an interesting variety thus derived. Red-eyed whites have been tested by crosses with reds and creams and have been shown conclusively to be the red-eyed representative of these varieties, such crosses having given 9 reds, 3 creams, 6 red-eyed whites and 6 albinos only. This red-eyed white demonstrates most forcibly the complete inhibition of yellow in the presence of the red-eye factor.

In the albino condition black disappears from the coat except in patches on the nose, ears and feet, and occasionally some sootiness on the back. In this connection it is interesting to note that nose, ears and feet are gener-

ally the most highly pigmented regions in the dilute and red-eye conditions. In the albinos, yellow disappears entirely, just as in the red-eyes. The eyes are pink, due to the loss of all pigment from the iris and retina. The blacks and golden agoutis are replaced by sooty albinos; the reds, by clear albinos; and the black-and-red tortoise-shells, by albinos in which nose, feet and ears are sooty or white, depending on the location of the spots.

The effects of the four allelomorphs on the appearance of eye and fur may be tabulated as follows:

Effects of	On Eye Color	On Black Fur	On Yellow Fur
Intensity	Black	Black	Red
Dilution	Black	Sepia	Cream
Red-eye	Red	Sepia	White
Albinism	Pink	White (sepia points)	White

It should be added that dilution of pigmentation may be produced by other factors than members of the albino series of allelomorphs. In the foregoing discussion such factors have been assumed to be absent. It may be said, however, that by starting with variations which owe their dilutions to factors which are independent of the albino series, doubly dilute varieties have been produced on introducing the dilution or red-eye allelomorphs of albinism. The effects of dilution, red-eye and albinism on brown pigment are parallel in all cases to their effects on black.

It should be said that the sepia (or "blue") due to the dilution factor, or to the red-eye factor, varies through a wide range which intergrades with black. Thus Castle (1905) and Sollas (1909) recognized that both intense and dilute forms of pigmentation occur commonly in guinea-pigs, but did not suggest any factorial explanation because of this intergrading. The inheritance of these fluctuations is at present under investigation.

If intensity, dilution, red-eye and albinism are allelomorphs, gametes should always carry one, but only one, of the four. Zygotes must always have two representa-

tives from the series, never more or less, which two may, of course, be alike. Thus with dominance in the order of increasing pigmentation intense guinea-pigs should be homozygous, or else carry recessive dilution, recessive red-eye or recessive albinism, but never more than one of these; dilute guinea-pigs should be homozygous, or carry recessive red-eye or recessive albinism, but never both; red-eyes should be either homozygous or carry recessive albinism; and finally albinos should always be homozygous and never have the power of transmitting intensity, dilution, or red-eye to their descendants. All of these types have been obtained and tested with results in harmony with expectation. In this paper only a few of the most critical crosses will be given, reserving a more detailed discussion for a later paper.

Red-eye, as mentioned before, has occurred only in the descendants of certain pigs brought from Peru. No albinos appeared in the pure stock. In the pure races, red-eye behaved as a simple recessive. Thus intense by intense gave 63 intense and 19 red-eye young, while red-eye by red-eye gave only red-eyes, 28 in number.

As red-eyes had never appeared in our stock guinea-pigs, it was natural to expect that any stock pig crossed with red-eye, an apparently recessive condition, would give only intense young. As a matter of fact, however, numerous red-eye young appeared in F_1. The next question was whether one kind of stock differed from another in its power of bringing about this apparent reversal of dominance. A study of the records soon showed that albinism had something to do with the matter, as the following tabulation indicates:

	Intense	Red-eye	Albino
Red-eye × intense	48	22	20
Red-eye × albino	0	80	32

The difference in result of the two sorts of matings is obviously significant. With an intense parent there were more than 50 per cent. intense young and comparatively few red-eye young. With an albino parent there were no

intense but numerous red-eye young. The intense animals and the albinos used were of the same stock and hence could differ consistently only as regards albinism. Clearly red-eye is not inherited independently of albinism. That the apparent reversal of dominance is not due merely to the presence of recessive albinism is shown by the fact that F_1 red-eyes crossed *inter se* produced 89 red-eyes, 36 albinos, but no intense young. Among these F_2 red-eyes, some were demonstrated to be free from recessive albinism. This complete inability of albinos to transmit the intense allelomorph of red-eye in crosses with the latter can only be interpreted in one way, aside from linkage hypotheses. The intense allelomorph of red-eye must also be an allelomorph of albinism. Thus red-eye must either be an allelomorph of albinism or be albinism itself, genetically, plus a modifying factor. The latter rather improbable hypothesis has been definitely eliminated. The pure South American stock under this hypothesis must be homozygous for all such modifying factors, since no albinos have appeared among them. The hypothetical modifying factor must be a dominant unit factor to account for the results given for F_1 and F_2.

Some pure South American intense animals were crossed with stock albinos. They produced F_1 intense young, which must (under the hypothesis) be heterozygous for albinism and for the modifying factor. These F_1 intense young were back-crossed with stock albinos. There should be both red-eyes and albinos among the young, 25 per cent. of each, if red-eye is albinism associated with a modifier. But no red-eyes actually appeared. There were 14 intense and 25 albinos. The chance that no red-eyes would appear in 39 young is $(3/4)^{39}$ or .000,001. Thus the hypothesis that red-eye is albinism plus a modifier may be dismissed.

On the view that red-eye is a dominant allelomorph of albinism, the results above are easily explained. Red-eye by albino or by red-eye should give only red-eyes and albinos. The F_1 intense, which must carry recessive al-

binism because of the stock albino parent, should not be able to transmit red-eye. As for hypotheses of linkage, it need only be said that no results have been obtained which require them. The critical crosses have all been made reciprocally as regards sex.

The fact that dilutes are more or less intermediate between intense and red-eye varieties suggested the following experiments which were designed to demonstrate at once whether there was any relation in inheritance between albinism and dilution. Dilutes were crossed with albinos from certain stocks which for years had given only intense and albino young, but no dilutes. Second, intense pigs from these same stocks, which had given only intense and albino young, were crossed with albinos from dilute stock. If intensity and dilution form a pair of allelomorphs which segregate independently of the pair, color and albinism (as is the case in mice and rabbits), these two crosses must give identical results. In each case color is introduced by one parent, albinism by the other; the intensity of certain stocks by one parent, the dilution of certain stocks by the other parent. In fact identical results should be obtained regardless of whether dilution is due to a unit factor or multiple factors, or even whether its inheritance is Mendelian or not, provided only that it is inherited independently of albinism. As it happened, these two crosses gave strikingly different results. The first cross, viz., dilute by albino from intense stock, gave only dilutes, 37 in number, aside from albinos. The second cross, intense from intense stock by albino from dilute stock, gave only intense young, 49 in number, aside from albinos. These different results can only be explained by assuming that a member of the albino series of allelomorphs, recessive to intensity, is essential to dilute animals. Thus genetically, dilution may be albinism plus a modifying factor, or it may be red-eye plus a modifying factor, or it may be a new allelomorph. The last has proved to be the case. A large number of intense animals, at least half of which under

the first hypothesis would be expected to be heterozygous both for albinism and the modifying factor, have been crossed with albinos. None of them have given both albinos and dilutes. It is of course expected under the hypothesis of allelomorphism that no intense animal should transmit both albinism and dilution. The view that dilution is red-eye plus a modifying factor has also been eliminated. Red-eye has never appeared in our stock guinea-pigs, which must therefore be pure for any modifying factor which might change a red-eye to a dilute. But red-eyes of any generation crossed with stock albinos have never given dilutes in 105 young. Therefore dilution can not be red-eye plus a modifying factor. The only remaining hypothesis is that dilution is an allelomorph of red-eye and albinism. It is dominant to both since dilute by dilute has often given red-eyes and albinos, while the latter varieties crossed *inter se* have never given dilutes.

Thus we have four allelomorphs corresponding to four grades of pigmentation. The existence of such series has a bearing on the nature of unit factors. The results could be explained by perfect coupling, but such an explanation seems highly arbitrary where the characters fall into a natural physiological series. The series seems to suggest that we have four variations in some one entity. Furthermore, while we have only four such variations at the Bussey Institution, it may well be that others exist elsewhere, forming perhaps a continuous series. Such variations in this factor probably do not occur frequently. When they do occur they probably take place by distinct steps. The rather frequent occurrence of albinos in wild species, without intermediates, indicates that variation from one extreme to the other in the condition of the factor may take place.

A point which has a bearing on the physiology of pigment is the fact that members of the albino series of allelomorphs do not cause diminution in quantity of pigment, merely as pigment, but affect yellow pigment differently from black. This is seen most clearly in the red-eyes, in

which yellow is completely inhibited, while black is only slightly affected.

Finally the question is raised whether anything similar to this can be found among other mammals. Albinos are found in many mammals as well as in lower animals. As to multiple allelomorphs, the case of the Himalayan rabbit is well known and compares well with the guinea-pig series. The Himalayan rabbit with its pink eyes and white fur with dark patches on nose, ears and feet is comparable to the guinea-pig albino. The complete albino rabbit recessive to the Himalayan is lower in the series than anything known in guinea-pigs. The dilution of the blue rabbit as well as that of the blue mouse and maltese cat is of a different type from the guinea-pig dilution. As Miss Sollas has shown, the pigment is clumped instead of uniformly decreased in quantity. The effect is slate-blue instead of sepia-brown. Mr. H. D. Fish has made crosses (unpublished data, to which I refer with his permission) which show as expected that rabbit dilution is inherited wholly independently of albinism.

In man, we have albinos which are probably comparable to guinea-pig and rabbit albinos. A study of the enormous collection of data in the Monograph of Pearson, Nettleship and Usher convinces one that albinism in man is recessive. But as Pearson points out, there are many grades of albinism and each grade tends to maintain its identity in inheritance. Among negroes there are albinos with blue irises, red pupils, white skin and nearly white hair. There are also darker grades, as with brown skin, eyes and hair. There is no sharp line anywhere between the complete albinos and the so-called xanthous types. In the white races albinos pass into the extreme blonds in a continuous series. In fact, study of records convinces one that in some cases the same factor may produce well-marked albinism, with red eyes, nystagmus and photophobia in one member of a family, but merely extreme blondism in another. It is worthy of note that human light hair resembles closely the sepia of dilute guinea-

pigs, and not at all the slate blue of dilute rabbits, mice and cats. Thus, while I have not been able to find any critical evidence, the suggestion seems worth making that a series of allelomorphs of albinism may be in part responsible for differences in intensity of human pigmentation.

Summing up: the results in guinea-pigs and rabbits suggest that there is a hereditary factor in mammals, which may exist with stability at different stages of divergence from the normal; that divergence from the normal in the factor tends to produce in the animal a corresponding reduction in the quantity of melanin pigment throughout the body, conspicuously in fur, skin and eyes, of which reduction the limit is complete albinism; that in this reduction the qualitative differentiation of the pigment is a factor, in that yellow pigment is affected more strongly than black and its threshold of complete inhibition is reached with less divergence of the factor; that in the reduction the location of the pigment is a factor, in that there is less tendency toward reduction at the extremities—feet, ears and nose—than elsewhere in skin and fur; that, finally, any stage of divergence is dominant to any stage more remote from the normal.

In conclusion, I wish to thank Professor Castle for the opportunity to carry on this work and for numerous suggestions during its progress.

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PROGRESSIVE EVOLUTION AND THE ORIGIN OF SPECIES[1]

PROFESSOR ARTHUR DENDY

THE opening years of the present century have witnessed a remarkable development of biology as an experimental science, a development which, however full of promise it may be for the future, for the time being appears to have resulted in a widespread disturbance of ideas which have themselves only recently succeeded in gaining general acceptance. The theory of organic evolution, plainly enough enunciated at the close of the eighteenth and the beginning of the nineteenth century by Buffon, Lamarck, and Erasmus Darwin, remained unconvincing to the great majority of thinking men until the genius of Charles Darwin not only brought together and presented the evidence in such a manner that it could no longer be ignored, but elaborated a logical explanation of the way in which organic evolution might be supposed to have taken place. Thanks to his labors and those of Alfred Russel Wallace, supported by the powerful influence of such men as Huxley and Hooker, the theory was placed upon a firm foundation, in a position which can never again be assailed with any prospect of success.

This statement is, I believe, entirely justified with regard to the theory of organic evolution itself, but the case is very different when we come to investigate the position of the various subsidiary theories which have been put forward from time to time with regard to what may perhaps be termed the *modus operandi,* the means by which organic evolution has been effected. It is in this field that controversy rages more keenly than ever before. Lamarck told us that evolution was due to the

[1] Address of the president of the section of zoology, British Association for the Advancement of Science, Australia, 1914.

accumulated results of individual effort in response to a changing environment, and also to the direct action of the environment upon the organism. Darwin and Wallace taught us that species originated by the natural selection of favorable variations, and under the influence of Weismann's doctrine of the non-inheritance of acquired characters the theory of natural selection is in danger of becoming crystallized into an inflexible dogma. In recent years de Vries has told us that species arise by sudden mutations, and not by slow successive changes, while one of the most extreme exponents of "Mendelism," Professor Lotsy, lately informed us that all species arise by crossing, and seriously suggested that the vertebrate type arose by the crossing of two invertebrates!

This curious and many-sided divergence of opinion amongst expert biologists is undoubtedly largely due to the introduction of experimental methods into biological science. Such methods have proved very fruitful in results which at first sight seem to be mutually contradictory, and each group of workers has built up its own theory mainly on the basis of observations in its own restricted field.

Professor Bateson has said in his recently published "Problems of Genetics":

> When . . . we contemplate the problem of evolution at large the hope at the present time of constructing even a mental picture of that process grows weak almost to the point of vanishing. We are left wondering that so lately men in general, whether scientific or lay, were so easily satisfied. Our satisfaction, as we now see, was chiefly founded on ignorance.[2]

In view of this striking pronouncement on the part of one who has devoted his life with signal success to the experimental investigation of evolutionary problems, the remarks which I propose to lay before you for your consideration to-day may well appear rash and ill-advised. I cannot believe, however, that the position is really quite so black as it is painted. We must perforce admit that the divers theories with regard to the work-

[2] "Problems of Genetic," p. 97.

ing of organic evolution cannot all be correct in all their details, but it may be that each contains its own elements of truth, and that if these elements can but be recognized and sorted out, they may perhaps be recombined in such a form as to afford at any rate a plausible working hypothesis. We must bear in mind from the outset that in dealing with such a complex problem many factors have to be taken into account, and that widely different views on the question may be merely one-sided and not necessarily mutually exclusive.

I take it there are three principal facts, or groups of facts, that have to be accounted for by any theory of organic evolution:

1. The fact that, on the whole, evolution has taken place in a progressive manner along definite and divergent lines.

2. The fact that individual animals and plants are more or less precisely adapted in their organization and in their behavior to the conditions under which they have to live.

3. The fact that evolution has resulted in the existence on the earth to-day of a vast number of more or less well-defined groups of animals and plants which we call species.

The first of these facts appears to me to be the most fundamental, and at the same time the one to which least attention is usually paid. The great question, after all, is, Why do organisms progress at all instead of remaining stationary from generation to generation? To answer this question it is not necessary to go back to the beginning and consider the case of the first terrestrial organisms, whatever they may have been, nor are we obliged to take as illustrations the lowest organisms known to us as existing at the present day. We may consider the problem at any stage of evolution, for at each stage progress is, or may be, still taking place. We may even begin by considering what is usually regarded as the highest stage of all, man himself; and indeed this seems the most natural thing to do, for we

certainly know more about the conditions of progress in man than in any other organism. I refer, of course, at the moment, not to progress in bodily organization, but to progress in the ordinary sense of the word, the progress, say, of a family which rises in the course of a few generations from a position of obscure poverty to one of wealth and influence. You may perhaps say that such a case has no bearing upon the problem of organic evolution in a state of nature, and that we ought to confine our attention to the evolution of bodily structure and function. If so, I must reply that you have no right to limit the meaning of the term evolution in this manner; the contrast between man and nature is purely arbitrary; man is himself a living organism, and all the improvements that he effects in his own condition are part of the progress of evolution in his particular case. At any rate I must ask you to accept this case as our first illustration of a principle that may be applied to organisms in general.

If we inquire into the cause of the progress of our human family I think there can be only one answer—it is due to the accumulation of capital, or, as I should prefer to put it, to the accumulation of potential energy, either in the form of material wealth or of education. What one generation saves is available for the next, and thus each succeeding generation gets a better start in life, and is able to rise a little higher than the preceding one.

Every biologist knows, of course, that there are many analogous cases amongst the lower animals, and also amongst plants. The accumulation of food-yolk in the egg has undoubtedly been one of the chief factors in the progressive evolution of animals, although it has been replaced in the highest forms by a more effective method of supplying potential energy to the developing offspring. It may indeed be laid down as a general law that each generation, whether of animals or of plants, accumulates more energy than it requires for its own maintenance, and uses the surplus to give the next gen-

eration a start in life. There is every reason to believe that this has been a progressive process throughout the whole course of evolution, for the higher the degree or organization the more perfect do we find the arrangements for securing the welfare of the offspring.

We cannot, of course, trace this process back to its commencement, because we know nothing of the nature of the earliest living things, but we may pause for a moment to inquire whether any phenomena occur amongst simple unicellular organisms that throw any light upon the subject. What we want to know is—How did the habit of accumulating surplus energy and handing it on to the next generation first arise?

Students of Professor H. S. Jennings's admirable work on the "Behavior of the Lower Organisms" will remember that his experiments have led him to the conclusion that certain Protozoa, such as *Stentor*, are able to learn by experience how to make prompt and effective responses to certain stimuli; that after they have been stimulated in the same way a number of times they make the appropriate response at once without having to go through the whole process of trial and error by which it was first attained. In other words, they are able by practise to perform a given action with less expenditure of energy. Some modification of the protoplasm must take place which renders the performance of an act the easier the oftener it has been repeated. The same is, of course, true in the case of the higher animals, and we express the fact most simply by saying that the animal establishes habits. From the mechanistic point of view we might say that the use of the machine renders it more perfect and better adapted for its purpose. In the present state of our knowledge I think we cannot go beyond this, but must content ourselves with recognizing the power of profiting by experience as a fundamental property of living protoplasm.

It appears to me that this power of profiting by experience lies at the root of our problem, and that in it

we find a chief cause of progressive evolution. Jennings speaks of the principle involved here as the "Law of the readier resolution of physiological states after repetition," and, similarly, I think we must recognize a "Law of the accumulation of surplus energy" as resulting therefrom. Let us look at the case of the accumulation of food-yolk by the egg-cell a little more closely from this point of view. Every cell takes in a certain amount of potential energy in the form of food for its own use. If it leads an active life either as an independent organism or as a constituent part of an organism, it may expend by far the greater part, possibly even the whole, of that energy upon its own requirements, but usually something is left over to be handed down to its immediate descendants. If, on the other hand, the cell exhibits very little activity and expends very little energy, while placed in an environment in which food is abundant, it will tend to accumulate surplus energy in excess of its own needs. Such is the case with the egg-cells of the multicellular animals and plants. Moreover, the oftener the process of absorbing food-material is repeated the easier does it become; in fact, the egg-cell establishes a habit of storing up reserve material or food-yolk. Inasmuch as it is a blastogenic character, there can be no objection to the supposition that this habit will be inherited by future generations of egg-cells. Indeed we are obliged to assume that this will be the case, for we know that the protoplasm of each succeeding generation of egg-cells is directly continuous with that of the preceding generation. We thus get at any rate a possibility of the progressive accumulation of potential energy in the germ-cells of successive generations of multicellular organisms, and, of course, the same argument holds good with regard to successive generations of *Protista*.

It would seem that progressive evolution must follow as a necessary result of the law of the accumulation of surplus energy in all cases where there is nothing to

counteract that law, for each generation gets a better start than its predecessor, and is able to carry on a little further its struggle for existence with the environment. It may be said that this argument proves too much, that if it were correct all organisms would by this time have attained to a high degree of organization, and that at any rate we should not expect to find such simple organisms as bacteria and *Amebæ* still surviving. This objection, which, of course, applies equally to other theories of organic evolution, falls to the ground when we consider that there must be many factors of which we know nothing which may prevent the establishment of progressive habits and render impossible the accumulation of surplus energy. Many of the lower organisms, like many human beings, appear to have an inherent incapacity for progress, though it may be quite impossible for us to say to what that incapacity is due.

It will be observed that in the foregoing remarks I have concentrated attention upon the storing up of reserve material by the egg-cells, and in so doing have avoided the troublesome question of the inheritance of so-called acquired characters. I do not wish it to be supposed, however, that I regard this as the only direction in which the law of the accumulation of surplus energy can manifest itself, for I believe that the accumulation of surplus energy by the body may be quite as important as a factor in progressive evolution as the corresponding process in the germ-cells themselves. The parents, in the case of the higher animals, may supply surplus energy, in the form of nutriment or otherwise, to the offspring at all stages of its development, and the more capital the young animal receives the better will be its chances in life, and the better those of its own offspring.

In all these processes, no doubt, natural selection plays an important part, but, in dealing with the accumulation of food material by the egg-cells, one of my objects has been to show that progressive evolution would take place

even if there were no such thing as natural selection, that the slow successive variations in this case are not chance variations, but due to a fundamental property of living protoplasm and necessarily cumulative.

Moreover, the accumulation of surplus energy in the form of food-yolk is only one of many habits which the protoplasm of the germ-cells may acquire in a cumulative manner. It may learn by practise to respond with increased promptitude and precision to other stimuli besides that of the presence of nutrient material in its environment. It may learn to secrete a protective membrane, to respond in a particular manner to the presence of a germ-cell of the opposite sex, and to divide in a particular manner after fertilization has taken place.

Having thus endeavored to account for the fact that progressive evolution actually occurs by attributing it primarily to the power possessed by living protoplasm of learning by experience and thus establishing habits by which it is able to respond more quickly to environmental stimuli, we have next to inquire what it is that determines the definite lines along which progress manifests itself.

Let us select one of these lines and investigate it as fully as the time at our disposal will permit, with the view of seeing whether it is possible to formulate a reasonable hypothesis as to how evolution may have taken place. Let us take the line which we believe has led up to the evolution of air-breathing vertebrates. The only direct evidence at our disposal in such a case is, of course, the evidence of paleontology, but I am going to ask you to allow me to set this evidence, which, as you know, is of an extremely fragmentary character, aside, and base my remarks upon the ontogenetic evidence, which, although indirect, will, I think, be found sufficient for our purpose. One reason for concentrating our attention upon this aspect of the problem is that I wish to show that the recapitulation of phylogenetic history in individual development is a logical necessity if evolution has really taken place.

We may legitimately take the nucleated Protozoon cell as our starting point, for, whatever may have been the course of evolution that led up to the cell, there can be no question that all the higher organisms actually start life in this condition.

We suppose, then, that our ancestral Protozoon acquired the habit of taking in food material in excess of its own requirements, and of dividing into two parts whenever it reached a certain maximum size. Here again we must, for the sake of simplicity, ignore the facts that even a Protozoon is by no means a simple organism, and that its division, usually at any rate, is a very complicated process. Each of the daughter-cells presently separates from its sister-cell and goes its own way as a complete individual, still a Protozoon. It seems not improbable that the separation may be due to the renewed stimulus of hunger, impelling each cell to wander actively in search of food. In some cases, however, the daughter-cells remain together and form a colony, and probably this habit has been rendered possible by a sufficient accumulation of surplus energy in the form of food-yolk on the part of the parent rendering it unnecessary for the daughter-cells to separate in search of food at such an early date. One of the forms of colony met with amongst existing Protozoa is the hollow sphere, as we see it, for example, in *Sphærozoum* and *Volvox,* and it is highly probable that the assumption of this form is due largely, if not entirely, to what are commonly called mathematical causes, though we are not in a position to say exactly what these causes may be. The widespread occurrence of the blastosphere or blastula stage in ontogeny is a sufficiently clear indication that the hollow, spherical Protozoon colony formed a stage in the evolution of the higher animals.

By the time our ancestral organism has reached this stage, and possibly even before, a new complication has arisen. The cells of which the colony is composed no longer remain all alike, but become differentiated, pri-

marily into two groups, which we distinguish as somatic-cells and germ-cells respectively.

From this point onwards evolution ceases to be a really continuous process, but is broken up into a series of ontogenies, at the close of each of which the organism has to go back and make a fresh start in the unicellular condition, for the somatic cells sooner or later become exhausted in their conflict with the environment and perish, leaving the germ-cells behind to take up the running. That the germ-cells do not share the fate of the somatic cells must be attributed to the fact that they take no part in the struggle for existence to which the body is exposed. They simply multiply and absorb nutriment under the protection of the body, and therefore retain their potential energy unimpaired. They are in actual fact, as is so often said, equivalent to so many protozoa, and, like the protozoa, are endowed with a potential immortality.

We know that, if placed under suitable conditions, or in other words, if exposed to the proper environmental stimuli, these germ-cells will give rise to new organisms, like that in the body of which they were formerly enclosed. One of the necessary conditions is, with rare exceptions, the union of the germ-cells in pairs to form zygotes or fertilized ova; but I propose, in the first instance, for the sake of simplicity, to leave out of account the existence of the sexual process and the results that follow therefrom, postponing the consideration of these to a later stage of our inquiry. I wish, moreover, to make it quite clear that organic evolution must have taken place if no such event as amphimixis had ever occurred.

What, then, may the germ-cells be expected to do? How are they going to begin their development? In endeavoring to answer this question we must remember that the behavior of an organism at any moment depends upon two sets of factors—the nature of its own constitution on one hand, and the nature of its environ-

ment on the other. If these factors are identical for any two individual organisms, then the behavior of these two individuals must be the same. If the germ-cells of any generation are identical with those of the preceding generation, and if they develop under identical conditions, then the soma of the one generation must also be identical with that of the other.[3] Inasmuch as they are parts of the same continuous germ-plasm—leaving out of account the complications introduced by amphimixis—we may assume that the germ-cells of the two generations are indeed identical in nearly every respect; but there will be a slight difference, due to the fact that those of the later generation will have inherited a rather larger supply of initial energy and a slightly greater facility for responding to stimuli of various kinds, for the gradual accumulation of these properties will have gone a stage further. The environment also will be very nearly identical in the two cases, for we know from experiment that if it were not the organism could not develop at all.

Throughout the whole course of its ontogeny the organism must repeat with approximate accuracy the stages passed through by its ancestors, because at every stage there will be an almost identical organism exposed to almost identical stimuli. We may, however, expect an acceleration of development and a slight additional progress at the end of ontogeny as the result of the operation of the law of the accumulation of surplus energy and of the slightly increased facility in responding to stimuli. The additional progress, of course, will probably be so slight that from one generation to the next we should be quite unable to detect it, and doubtless there will be frequent backslidings due to various causes.

We can thus formulate a perfectly reasonable explanation of how it is that the egg first undergoes segmentation and then gives rise to a blastula resembling a hol-

[3] This is, of course, a familiar idea. Compare Driesch, "Gifford Lectures," 1907, p. 214.

low protozoon colony; it does so simply because at every stage it must do what its ancestors did under like conditions. We can also see that progressive evolution must follow from the gradual accumulation of additions at the end of each ontogeny, these additions being rendered possible by the better start which each individual gets at the commencement of its career.

Let us now glance for a moment at the next stage in phylogeny, the conversion of the hollow spherical protozoon colony into the cœlenterate type of organization, represented in ontogeny by the process of gastrulation. Here again it is probable that this process is explicable to a large extent upon mechanical principles. According to Rhumbler,[4] the migration of endoderm cells into the interior of the blastula is partly due to chemotaxis and partly to changes of surface tension, which decreases on the inner side of the vegetative cells owing to chemical changes set up in the blastocœl fluid.

We may, at this point, profitably ask the question, Is the endoderm thus formed an inherited feature of the organism? The material of which it is composed is, of course, derived from the egg-cell continuously by repeated cell-division, but the way in which that material is used by the organism depends upon the environment, and we know from experiment that modifications of the environment actually do produce corresponding modifications in the arrangement of the material. We know, for example, that the addition of salts of lithium to the water in which certain embryos are developing causes the endoderm to be protruded instead of invaginated, so that we get a kind of inside-out gastrula, the well-known lithium larva.

It appears, then, than an organism really inherits from its parents two things: (1) a certain amount of protoplasm loaded with potential energy, with which to begin operations, and (2) an appropriate environment. Ob-

[4] Quoted by Przibram, "Experimental Zoology," English Trans., Part I, p. 47.

viously the one is useless without the other. An egg can not develop unless it is provided with the proper environment at every stage. Therefore, when we say that an organism inherits a particular character from its parents, all we mean is that it inherits the power to produce that character under the influence of certain environmental stimuli.[5] The inheritance of the environment is of at least as much importance as the inheritance of the material of which the organism is composed. The latter, indeed, is only inherited to a very small extent, for the amount of material in the egg-cell may be almost infinitesimal in comparison with the amount present in the adult, nearly the whole of which is captured from the environment and assimilated during ontogeny.

From this point of view the distinction between somatogenic and blastogenic characters really disappears, for all the characters of the adult organism are acquired afresh in each generation as a result of response to environmental stimuli during development. This is clearly indicated by the fact that you cannot change the stimuli without changing the result.

Time forbids us to discuss the phylogenetic stages through which the cœlenterate passed into the cœlomate type, the cœlomate into the chordate, and the chordate into the primitive vertebrate. We must admit that as yet we know nothing of the particular causes that determined the actual course of evolution at each successive stage. What we do know, however, about the influence of the environment, both upon the developing embryo and upon the adult, is sufficient to justify us in believing that every successive modification must have been due to a response on the part of the organism to some environmental change. Even if the external conditions remained practically identical throughout long periods of time, we must remember that the internal conditions would be different in each generation, because

[5] Compare Dr. Archdall Reid's suggestive essay on "Biological Terms" (*Bedrock*, January, 1914).

each generation starts with a slightly increased capital and carries on its development a little further under internal conditions modified accordingly.

At this point it may be asked, Is the response to environmental stimuli a purely mechanical one, and, if so, how can we account for the fact that at every stage in its evolution the organism is adapted to its environment? We shall have to return to this question later on, but it may be useful to point out once more that there is good reason to believe—especially from the experimental work of Jennings—that the response of even a unicellular organism to stimuli is to a large extent purposive; that the organism learns by experience, by a kind of process of trial and error, how to make the response most favorable to itself under any given change of conditions; in other words, that the organism selects those modes of response that are most conducive to its own well-being. Under the term response to stimuli we must, of course, include those responses of the living protoplasm which result in modifications of bodily structure, and hence the evolution of bodily structure will, on the whole, be of an adaptive character and will follow definite lines. There is good reason for believing, however, that many minor modifications in structure may arise and persist, incidentally as it were, that have no significance as adaptations.

One of the most remarkable and distinctive features of the lower vertebrates is the presence of gill-slits as accessory organs of respiration. These gill-slits are clearly an adaptation to aquatic life. When the ancestors of the higher vertebrates left the water and took to life on land the gills disappeared and were replaced by lungs, adapted for air-breathing. The change must, of course, have been an extremely gradual one, and we get a very clear indication of how it took place in the surviving dipnoids, which have remained in this respect in an intermediate condition between the fishes and the amphibia, possessing and using both gills and lungs.

We also know that even the most highly specialized air-breathing vertebrates, which never live in water and never require gills or gill-slits at all, nevertheless possess very distinct gill-slits during a certain period of their development. This is one of the most familiar illustrations of the law of recapitulation, and my only excuse for bringing it forward now is that I wish, before going further, to consider a difficulty—perhaps more apparent than real—that arises in connection with such cases.

It might be argued that if gill-slits arose in response to the stimuli of aquatic life, and if these stimuli are no longer operative in the case of air-breathing vertebrates, then gill-slits ought not to be developed at any stage of their existence. This argument is, I think, fully met by the following considerations.

At any given moment of ontogenetic development the condition of any organ is merely the last term of a series of morphogenetic stages, while its environment at the same moment—which, of course, includes its relation to all the other organs of the body—is likewise merely the last term of a series of environmental stages. We have thus two parallel series of events to take into consideration in endeavoring to account for the condition of any part of an organism—or of the organism as a whole—at any period of its existence:

$$E_1\ E_2\ E_3\ \ldots\ E_n \text{ environmental stages,}$$
$$M_1\ M_2\ M_3\ \ldots\ M_n \text{ morphogenetic stages.}$$

Ontogeny is absolutely conditioned by the proper correlation of the stages of these two series at every point, and hence it is that any sudden change of environment is usually attended by disastrous consequences. Thus, after the fish-like ancestors of air-breathing vertebrates had left the water and become amphibians, they doubtless still had to go back to the water to lay their eggs, in order that the eggs might have the proper conditions for their development.

Obviously the environment can only be altered with extreme slowness, and one of the first duties of the parent is to provide for the developing offspring conditions as nearly as possible identical with those under which its own development took place. It is, however, inevitable that, as phylogenetic evolution progresses, the conditions under which the young organism develops should change. In the first place, the mere tendency to acceleration of development, to which we have already referred, must tend to dislocate the correlation between the ontogenetic series and the environmental series. Something of this kind seems to have taken place in the life-cycle of many hydrozoa, resulting in the suppression of the free medusoid generation and the gradual degeneration of the gonophore. But it is probably in most cases change in the environment of the adult that is responsible for such dislocation.

To return to the case of the amphibians. At the present day some amphibians, such as the newts and frogs, still lay their eggs in water, while the closely related salamanders retain them in the oviducts until they have developed into highly organized aquatic larvæ, or even what is practically the adult condition. Kammerer has shown that the period at which the young are born can be varied by changing the environment of the parent. In the absence of water the normally aquatic larvæ of the spotted salamander may be retained in the oviduct until they have lost their gills, and they are then born in the fully-developed condition, while, conversely, the alpine salamander, of which the young are normally born in the fully-developed state, without gills, may be made to deposit them prematurely in water in the larval, gill-bearing condition.

There can be no doubt that the ancestral amphibians laid their eggs in water in a completely undeveloped condition. The habit of retaining them in the body during their development must have arisen very gradually in the phylogenetic history of the salamanders, the period

for which the young were retained growing gradually longer and longer. It is obvious that this change of habit involves a corresponding change in the environmental conditions under which the young develop, and in cases in which the young are not born until they have reached practically the adult condition this change directly affects practically the whole ontogeny. We may say that the series

$$E_1\ E_2\ E_3\ \ldots\ E_n \text{ has become}$$
$$E_1'\ E_2'\ E_3'\ \ldots\ E_n',$$

and as the change of environment must produce its effect upon the developing organism the series

$$M_1\ M_2\ M_3\ \ldots\ M_n \text{ will have become}$$
$$M_1'\ M_2'\ M_3'\ \ldots\ M_n'.$$

We must remember that throughout the whole course of phylogenetic evolution this series is constantly lengthening, so that what was the adult condition at one time becomes an embryonic stage in future generations, and the series thus represents not only the ontogeny, but also, though in a more or less imperfect manner, the phylogeny of the organism.

The character of each stage in ontogeny must depend upon (1) the morphological and physiological constitution of the preceding stage, and (2) the nature of the environment in which development is taking place. We can not, however, distinguish sharply between those two sets of factors, for, in a certain sense, the environment gradually becomes incorporated in the organism itself as development proceeds, each part contributing to the environment of all the remainder, and the influence of this internal portion of the environment ever becoming more and more important.

The whole process of evolution depends upon changes of environment taking place so gradually that the necessary self-adjustment of the organism at every stage is possible. In the case of our amphibia the eggs could

possibly undergo the first stages of development, the preliminary segmentation, within the oviduct of the parent just as well as in the water, for in both cases they would be enclosed in their envelopes, and the morphological differences between the early stages in the two cases might be expected to be quite insignificant. But it must be the same at each term of the series, for each term is built upon the foundation of the preceding one, and the whole process takes place by slow and imperceptible degrees.

It is true that by the time we reach the formation of the vestigial gill-slits in the embryo of one of the higher vertebrates the environmental conditions are very different from those under which gill-slits were developed in their aquatic ancestors. But what then? Are not the gill-slits also very different? The changed environment has had its effect. The gills themselves are never developed, and the gill-slits never become functional; moreover, they disappear completely at later stages of development, when the conditions of life become still more different and their presence would be actually detrimental to their possessor. The embryo with the vestigial gill-slits is, as a whole, perfectly well adapted to its environment, though the gill-slits themselves have ceased to be adaptive characters. They still appear because the environmental conditions, and especially the internal conditions, which have now become far more important than the external ones, are still such as to cause them to do so.

I think the chief difficulty in forming a mental picture of the manner in which evolution has taken place, and especially in accounting for the phenomenon of recapitulation in ontogeny, which is merely another aspect of the same problem, arises from attempting to take in too much at once. There is no difficulty in understanding how any particular stage is related to the corresponding stage in the previous generation, and the whole series of stages, whether looked at from the ontogenetic or from

the phylogenetic point of view, can be nothing else but the sum of its successive terms.

It will be convenient, before going further, to sum up the results at which we have so far arrived from the point of view of the theory of heredity. We have as yet seen no reason to distinguish between somatogenic and blastogenic characters. All the characters of the adult animal are acquired during ontogeny as the result of the reaction of the organism to environmental stimuli, both internal and external. All that the organism actually inherits is a certain amount of protoplasm—endowed with a certain amount of energy—and a certain sequence of environmental conditions. In so far as these are identical in any two successive generations the final result must be identical also, the child must resemble the parent; in so far as they are different the child will differ from the parent, but the differences in environment can not be very great without preventing development altogether.

So far, it is clear, there has been no need to think of the germ-cells as the bearers of material factors or determinants that are responsible for the appearance of particular characters in the adult organism; nor yet to suppose that they are, to use the phraseology of the mnemic theory of heredity, charged with the memories of past generations. They have been regarded as simple protoplasmic units, and the entire ontogeny has appeared as the necessary result of the reaction between the organism and its environment at each successive stage of development. This can not, however, be a complete explanation of ontogeny, for if it were we should expect all eggs, when allowed to develop under the same conditions from start to finish, to give rise to the same adult form, and this we know is not the case. We know also, from observation and experiment, that the egg is in reality by no means a simple thing but an extremely complex one, and that different parts of the egg may be definitely correlated with corresponding parts of the adult body. It

has been demonstrated in certain cases that the egg contains special organ-forming substances definitely located in the cytoplasm, and that if these are removed definite parts of the organism into which the egg develops will be missing. We know, also, that the nucleus of the germ-cell of either sex contains—at any rate, at certain periods—a number of perfectly well-defined bodies, the chromosomes, and these also have been definitely correlated in certain cases with special features of the adult organization.

Before we can hope to complete our mental picture of the manner in which organic evolution has taken place, if only in outline, it is evident that we must be able to account for the great complexity of structure which the germ-cells themselves have managed to acquire, and also to form some idea of the effect of this complication upon the development of both the individual and the race.

We must consider the origin of cytoplasmic and nuclear complications of the egg separately, for they appear to be due fundamentally to two totally distinct sets of factors. In the first place we have to remember that during oogenesis the egg-cell grows to a relatively large size by absorbing nutrient material from the body in which it is enclosed. It is this nutrient material that is used for building up the deutoplasm or food-yolk. There is good reason for believing that the character of this nutrient material will change, during the course of evolution, *pari passu* with the changing character of the organism by which it is supplied. Doubtless the change is of a chemical nature, for we know from precipitin experiments that the body fluids of closely allied species, or even of the two sexes of the same species, do exhibit distinctly recognizable differences in chemical composition. It also appears highly probable, if not certain, from such experiments as those of Agar upon *Simocephalus,* that substances taken in with the food, which bring about conspicuous modifications of bodily structure, may at the same time be absorbed and stored up by the egg-cells

so as to bring about corresponding changes in the adults into which the eggs develop.

There seems therefore to be no great difficulty in comprehending, at any rate in a general way, how the egg may become the repository of definite chemical substances, organ-forming substances if we like to call them so, possibly to be classed with the hormones and enzymes, which will influence the development in a particular manner as soon as the appropriate conditions arise.

Unfortunately, time will not allow of our following up this line of thought on the present occasion, but we may notice, before passing on, that with the accumulation of organ-forming substances in the egg we have introduced the possibility of changes in bodily structure, to whatever cause they may be due, being represented by correlated modifications in the germ-cells, and this is doubtless one of the reasons why the germ-cells of different animals are not all alike with regard to their potentialities of development.[6]

We now come to the question of how the nucleus of the germ-cell acquired its great complexity of structure. We are not concerned here with the origin of the differentiation into nucleus and cytoplasm and the respective parts played by the two in the life of the cell. The problem which we have to consider is the complication introduced by the sexual process, by the periodically recurring union of the germ-cells in pairs, or, as Weismann has termed it, amphimixis. This is well known to be essentially a nuclear phenomenon, in which the so-called chromatin substance is especially concerned, and it is a phenomenon which must have made its appearance at a very early stage of evolution, for it is exhibited in essentially the same manner alike in the higher plants and animals and in unicellular organisms.

Let us suppose, for the sake of argument, that when amphimixis first took place the chromatin of each germ-

[6] Compare Cunningham's "Hormone Theory of Heredity" (*Archiv für Entwicklungsmechanik der Organismen*, Bd. XXVI, Heft 3).

cell was homogeneous, but that it differed slightly in different germ cells of the same species as a result of exposure to slightly different conditions during its past history. What would be likely to happen when two different samples of chromatin came together in the zygote? The result would surely depend upon the interaction of the complex colloidal multimolecules of which the chroatin is composed. Various possibilities would arise. (1) The two samples might differ in such a way as to act as poisons to one another, disturbing each other's molecular equilibrium to such an extent that neither could survive. This is possibly what happens when an ovum is fertilized by a spermatozoon of a distinct species, though there are, of course, exceptions. (2) They might be so alike as to be able to amalgamate more or less completely, so that there would simply be an increase of chromatin of possibly more or less modified constitution. (3) They might continue to exist side by side, each maintaining its own individual character.

In the third case the union of the two different samples would give rise to a mass of chromatin of twofold nature, and repetition of the process from generation to generation would, as Weismann has shown, result in ever-increasing heterogeneity, until the chromatin came to consist of a great number of different concrete particles, each of which might conceivably differ from all the others. But when two heterogeneous masses of chromatin meet in the zygote there may be all sorts of mutual attractions and repulsions between the different colloidal multimolecules, for all three of our supposed cases may arise simultaneously, and thus the results may become extremely complicated.

The chromatin of the germ-cells in all existing organisms is undoubtedly heterogeneous, and this heterogeneity may be to some extent visibly expressed in its arrangement in more or less multiform chromosomes during mitosis. We may provisionally accept Weismann's view that these chromosomes are themselves

heterogeneous, being composed of chromomeres or ids, which in their turn are composed of determinants.

All this complexity of structure may be attributed to the effects of oft-repeated amphimixis, a view which is supported in the most striking manner by the fact that the nucleus in all ordinary somatic cells (in animals and in the diploid generation of plants) has a double set of chromosomes, one derived from the male and the other from the female parent, and by the well-known phenomenon of chromatin reduction which always precedes amphimixis.

When we approach the problem of heredity from the experimental side we get very strong evidence of the existence in the germ-plasm of definite material substances associated with the inheritance of special characters. Mendelian workers generally speak of these substances as factors, but the conception of factors is evidently closely akin to that of Weismann's hypothetical determinants. The cytological evidence fits in very well with the view that the factors in question may be definite material particles and it is quite possible that such particles may have a specific chemical constitution to which their effects upon the developing organism are due.

From our point of view the interesting thing is the possibility that arises through the sexual process of the permutation and combination of different factors derived from different lines of descent. A germ-cell may receive additions to its collection of factors or be subject to subtractions therefrom, and in either case the resulting organism may be more or less conspicuously modified.

By applying the method of experimental hybridization a most fruitful and apparently inexhaustible field of research has been opened up in this direction, in the development of which no one has taken a more active part than the present President of the British Association. There can not be the slightest doubt that a vast number of characters are inherited in what is called the Mende-

ian manner, and, as they are capable of being separately inherited and interchanged with others by hybridization, we are justified in believing that they are separately represented in the germ-cells by special factors. Important as this result is, I believe that at the present time there exists a distinct danger of exaggerating its significance. The fact that many new and apparently permanent combinations of characters may arise through hybridization, and that the organisms thus produced have all the attributes of what we call distinct species, does not justify us in accepting the grotesque view—as it appears to me—that all species have arisen by crossing, or even the view that the organism is entirely built up of separately transmissible "unit characters."

Bateson tells us that

> Baur has for example crossed species so unlike as *Antirrhinum majus* and *molle,* forms differing from each other in almost every feature of organization.

Surely the latter part of this statement can not be correct, for after all *Antirrhinum majus* and *molle* are both snapdragons, and exhibit all the essential characters of snapdragons.

I think it is a most significant fact that the only characters which appear to be inherited in Mendelian fashion are comparatively trivial features of the organism which must have arisen during the last stages of phylogeny. This is necessarily the case, for any two organisms sufficiently nearly related to be capable of crossing are identical as regards the vast majority of their characters. It is only those few points in which they differ that remain to be experimented on. Moreover, the characters in question appear to be all non-adaptive, having no obvious relation to the environment and no particular value in the struggle for existence. They are clearly what Weismann calls blastogenic characters, originating in the germ-plasm, and are probably identical with the mutations of de Vries. These latter are apparently chro-

matin-determined characters, for, as Dr. Gates has recently shown in the case of *Œnothera,* mutation may result from abnormal distribution of the chromosomes in the reduction division.[7]

We have next to inquire whether or not the Mendelian results are really in any way inconsistent with the general theory of evolution outlined in the earlier part of this address. Here we are obviously face to face with the old dispute between epigenesis and preformation. The theory of ontogeny which I first put forward is clearly epigenetic in character, while the theory of unit characters, represented in the germ-cells by separate "factors," is scarcely less clearly a theory of preformation, and of course the conception of definite organ-forming substances in the cytoplasm falls under the same category. The point which I now wish to emphasize is that the ideas of epigenesis and preformation are not not inconsistent with one another, and that, as a matter of fact, ontogenetic development is of a dual nature, an epigenesis modified by what is essentially preformation.

We have already dealt briefly with the question of organ-forming substances in the cytoplasm, and it must, I think, be clear that the existence of these is in no way incompatible with a fundamental epigenesis. We shall find directly that the same is true of Mendelian "factors" or Weismannian "determinants."

We have seen that it is possible to conceive of even a complex organism as inheriting nothing from its parent but a minute speck of protoplasm, endowed with potential energy, and a sequence of suitable environments, the interaction between the two bringing about a similar result in each suceeding generation, with a slow progressive evolution due to the operation of the law of accumulation of surplus energy. If any of the conditions of development are changed the result, as manifested in the organization of the adult, must undergo a corresponding modification. Suppose that the chromatin sub-

[7] *Quarterly Journal of Microscopical Science,* Vol. LIX, p. 557.

stance of the zygote is partially modified in molecular constitution, perhaps by the direct action of the environment, as appears to happen in the case of Tower's experiments on mutation in the potato beetle, or by the introduction of a different sample of chromatin from another individual by hybridization. What is the germ-plasm now going to do? When and how may the changes that have taken place in its constitution be expected to manifest themselves in the developing organism?

Let us consider what would be likely to happen in the first stages of ontogeny. If the germ-plasm had remained unaltered the zygote would have divided into blastomeres under the stimuli of the same conditions, both internal and external, as those under which the corresponding divisions took place in preceding generations. Is the presence of a number of new colloidal multimolecules in the germ-plasm going to prevent this? The answer to this question probably depends partly upon the proportion that the new multimolecules bear to the whole mass, and partly upon the nature of the modification that has taken place. If the existence of the new multimolecules is incompatible with the proper functional activity of the germ-plasm as a whole there is an end of the matter. The organism does not develop. If it is not incompatible we must suppose that the zygote begins its development as before, but that sooner or later the modification of the germ-plasm will manifest itself in the developing organism, in the first instance as a mutation. In cases of hybridization we may get a mixture in varying degrees of the distinguishing characters of the two parent forms, or we may get complete dominance of one form over the other in the hybrid generation, or we may even get some new form, the result depending on the mutual reactions of the different constituents of the germ-plasm.

The organism into which any zygote develops must be

a composite body deriving its blastogenic characters from different sources; but this cannot affect its fundamental structure, for the two parents must have been alike in all essential respects or they could not have interbred, and any important differences in the germ-plasm must be confined to the "factors" for the differentiating characters. The fundamental structure still develops epigenetically on the basis of an essentially similar germ-plasm and under essentially similar conditions as in the case of each of the two parents, and there is no reason to suppose that special "factors" have anything to do with it.

We thus see how new unit characters may be added by mutation and interchanged by hybridization while the fundamental constitution of the organism remains the same and the epigenetic course of development is not seriously affected. All characters that arise in this way must be regarded, from the point of view of the organism, as chance characters due to chance modifications of the germ-plasm, and they appear to have comparatively little influence upon the course of evolution.

One of the most remarkable features of organic evolution is that it results in the adaptation of the organism to its environment, and for this adaptation mutation and hybridization utterly fail to account. Of course the argument of natural selection is called in to get over this difficulty. Those organisms which happen to exhibit favorable mutations will survive and hand on their advantages to the next generation, and so on. It has frequently been pointed out that this is not sufficient. Mutations occur in all directions, and the chances of a favorable one arising are extremely remote. Something more is wanted, and this something, it appears to me, is to be found in the direct response of the organism to environmental stimuli at all stages of development, whereby individual adaptation is secured, and this individual adaptation must arise again and again in each succeeding

generation. Moreover, the adaptation must, as I pointed out before, tend to be progressive, for each successive generation builds upon a foundation of accumulated experience and has a better start than its predecessors.

Of course natural selection plays its part, as it must in all cases, even in the organic world, and I believe that in many cases—as, for example, in protective resemblance and mimicry—that part has been an extremely important one. But much more important than natural selection appears to me what Baldwin[8] has termed "Functional Selection," selection by the organism itself, out of a number of possible reactions, of just those that are required to meet any emergency. As Baldwin puts it, "It is the organism which secures from all its overproduced movements those which are adaptive and beneficial." Natural selection is here replaced by intelligent selection, for I think we must agree with Jennings[9] that we can not make a distinction between the higher and the lower organisms in this respect, and that all purposive reactions, or adjustments, are essentially intelligent.

Surely that much-abused philosopher, Lamarck, was not far from the truth when he said, "The production of a new organ in an animal body results from a new requirement which continues to make itself felt, and from a new movement which this requirement begets and maintains."[10] Is not this merely another way of saying that the individual makes adaptive responses to environmental stimuli? Where so many people fall foul of Lamarck is with regard to his belief in the inheritance of acquired characters. But in speaking of acquired characters Lamarck did not refer to such modifications as mutilations; he was obviously talking of the gradual self-adjustment of the organism to its environment.

[8] "Development and Evolution" (New York, 1902), p. 87.

[9] "Behavior of the Lower Organisms" (New York, 1906), pp. 334, 335.

[10] "Histoire naturelle des Animaux sans Vertèbres," Tom. I, 1815, p. 185.

We are told, of course, that such adjustments will only be preserved so long as the environmental stimuli by which they were originally called for continue to exercise their influence. Those who raise this objection are apt to forget that this is exactly what happens in evolution, and that the *sine qua non* of development is the proper maintenance of the appropriate environment, both internal and external. Natural selection sees to it that the proper conditions are maintained within very narrow limits.

A great deal of the confusion that has arisen with regard to the question of the inheritance of acquired characters is undoubtedly due to the quite unjustifiable limitation of the idea of "inheritance" to which we have accustomed ourselves. The inheritance of the environment is, as I have already said, just as important as the inheritance of the material foundation of the body, and whether or not a newly acquired character will be inherited must depend, usually at any rate, upon whether or not the conditions under which it arose are inherited. It is the fashion nowadays to attach very little imporance to somatogenic characters in discussing the problem of evolution. The whole fundamental structure of the body must, however, according to the epigenetic view, be due to the gradual accumulation of characters that arise as the result of the reactions of the organism to its environment, and are therefore somatogenic, at any rate in the first instance, though there is reason to believe that some of them may find expression in the germ-cells in the formation of organ-forming substances, and possibly in other ways. Blastogenic characters which actually originate in the germ-cells appear to be of quite secondary importance.

We still have to consider the question, How is it that organic evolution has led to the formation of those more or less well-marked groups of organisms which we call species? We have to note in the first place that there

is no unanimity of opinion amongst biologists as to what a species is. Lamarck insisted that nature recognizes no such things as species, and a great many people at the present day are, I think, still of the same opinion. In practise, however, every naturalist knows that there are natural groups to which the vast majority of individuals can be assigned without any serious difficulty. Charles Darwin maintained that such groups arose, under the influence of natural selection, through gradual divergent evolution and the extinction of intermediate forms. To-day we are told by de Vries that species originate as mutations which propagate themselves without alteration for a longer or shorter period, and by Lotsy that species originate by crossing of more or less distinct forms, though this latter theory leaves quite unsolved the problem of where the original forms that crossed with one another came from.

I think a little reflection will convince us that the origin of species is a different problem from that of the cause of progressive evolution. We can scarcely doubt, however, that Darwin was right in attributing prime importance to divergent evolution and the disappearance of connecting links. It is obvious that this process must give rise to more or less sharply separated groups of individuals to which the term species may be applied, and that the differences between these species must be attributed ultimately to differences in the response of the organism to differing conditions of the environment. It may be urged that inasmuch as different species are often found living side by side under identical conditions the differences between them can not have arisen in this way, but we may be quite certain that if we knew enough of their past history we should find that their ancestors had not always lived under identical conditions.

The case of flightless birds on oceanic islands is particularly instructive in this connection. The only satisfactory way of explaining the existence of such birds is

by supposing that their ancestors had well-developed wings, by the aid of which they made their way to the islands from some continental area. The conditions of the new environment led to the gradual disuse and consequent degeneration of the wings until they either became useless for flight or, in the case of the moas, completely disappeared. It would be absurd to maintain that any of the existing flightless birds are specifically identical with the ancestral flying forms from which they are descended, and it would, it appears to me, be equally absurd to suppose that the flightless species arose by mutation or by crossing, the same result being produced over and over again on different islands and in different groups of birds. This is clearly a case where the environment has determined the direction of evolution.

In such cases there is not the slightest ground for believing that crossing has had anything whatever to do with the origin of the different groups to which the term species is applied; indeed, the study of island faunas in general indicates very clearly that the *prevention* of crossing, by isolation, has been one of the chief factors in the divergence of lines of descent and the consequent multiplication of species, and Romanes clearly showed that even within the same geographical area an identical result may be produced by mutual sterility, which is the cause, rather than the result, of specific distinction.

Species, then, may clearly arise by divergent evolution under changing conditions of the environment, and may become separated from one another by the extinction of intermediate forms. The environmental stimuli (including, of course, the body as part of its own environment) may, however, act in two different ways: (1) Upon the body itself, at any stage of its development, tending to cause adaptation by individual selection of the most appropriate response; and (2) upon the germplasm, causing mutations or sudden changes, sports, in

fact, which appear to have no direct relation whatever to the well-being of the organism in which they appear, but to be purely accidental. Such mutations are, of course, inherited, and, inasmuch as the great majority of specific characters appear to have no adaptive significance, it seems likely that mutation has had a great deal to do with the origin of species, though it may have had very little to do with progressive evolution.

Similarly with regard to hybridization, we know that vast numbers of distinct forms, that breed true, may be produced in this way, but they are simply due to recombinations of mutational characters in the process of amphimixis, and have very little bearing upon the problem of evolution. If we like to call the new groups of individuals that originate thus "species," well and good, but it only means that we give that name, as a matter of convenience, to any group of closely related individuals which are distinguished by recognizable characters from the individuals of all other groups, and which hand on those characters to their descendants so long as the conditions remain the same. This, perhaps, is what we should do, and just as we have learned to regard individuals as the temporary offspring of a continuous stream of germ-plasm, so we must regard species as the somewhat more permanent but nevertheless temporary offshoots of a continuous line of progressive evolution. Individuals are to species what the germ-plasm is to individuals. One species does not arise from another species, but from certain individuals in that species, and when all the individuals become so specialized as to lose their power of adaptation, then changes in the environment may result in the extinction of that line of descent.

It is scarcely necessary to point out that no explanation that we are able to give regarding the causes of either phylogenetic or ontogenetic evolution can be complete and exhaustive. Science can never hope to get to

the bottom of things in any department of knowledge; there is always something remaining beyond our reach. If we are asked why an organism chooses the most appropriate response to any particular stimulus, we may suggest that this is the response that relieves it from further stimulation, but we cannot say how it learns to choose that response at once in preference to all others. If we are asked to account for some particular mutation, we may say that it is due to some modification in the constitution or distribution of the chromosomes in the germ-cells, but even if we knew exactly what that modification was, and could express it in chemical terms, we could not really say why it produces its particular result and no other, any more than the chemist can say why the combination of two gases that he calls oxygen and hydrogen gives rise to a liquid that he calls water.

There is one group of ontogenetic phenomena in particular that seems to defy all attempts at mechanistic interpretation. I refer to the phenomena of restitution, the power which an organism possesses of restoring the normal condition of the body after it has been violently disturbed by some external agent. The fact that a newt is able to regenerate its limbs over and over again after they have been removed, or that an echinoderm blastula may be cut in half and each half give rise to a perfect larva, is one of the most surprising things in the domain of biological science. We can not, at present, at any rate, give any satisfactory mechanistic explanation of these facts, and to attribute them to the action of some hypothetical entelechy, after the manner of Professor Hans Driesch, is simply an admission of our inability to do so. We can only say that in the course of its evolution each organism acquires an individuality or wholeness of its own, and that one of the fundamental properties of living organisms is to maintain that individuality. They are able to do this in a variety of ways, and can sometimes even replace a lost

organ out of material quite different from that from which the organ in question is normally developed, as in the case of the regeneration of the lens of the eye from the iris in the newt. That there must be some mechanism involved in such cases is, of course, self-evident, and we know that that mechanism may sometimes go wrong and produce monstrous and unworkable results; but it is, I think, equally evident that the organism must possess some power of directing the course of events, so as generally to secure the appropriate result; and it is just this power of directing chemical and physical processes, and thus employing them in its own interests, that distinguishes a living organism from an inanimate object.

In conclusion I ought, perhaps, to apologize for the somewhat dogmatic tone of my remarks. I must ask you to believe, however, that this does not arise from any desire on my part to dogmatize, but merely from the necessity of compressing what I wished to say into a totally inadequate space. Many years of patient work are still needed before we can hope to solve, even approximately, the problem of organic evolution, but it seemed to me permissible, on the present occasion, to indulge in a general survey of the situation, and see how far it might be possible to reconcile conflicting views and bring together a number of ideas derived from many sources in one consistent theory.

SHORTER ARTICLES AND DISCUSSION

THE ORIGIN OF A NEW EYE-COLOR IN DROSOPHILA REPLETA AND ITS BEHAVIOR IN HEREDITY

In September, 1913, a new eye-color "scarlet," appeared in one of my cultures of *Drosophila repleta* Wollaston. The new eye color is a bright scarlet when first hatched and darkens but little with age. The eyes of the wild flies, on the other hand, are a deep mahogany which darken soon after hatching until they are almost black. This last statement is true of the stocks I have found in New York City, Woods Hole, Mass., North Manchester, Ind., Brazil, Ind., and Terre Haute, Indiana. The eye-color of the newly emerged mutant corresponds to the color chart in Ridgeway's Color Guide, Plate VII, No. 11 (Boston, 1886). The large scarlet eye in contrast to the dark body of the fly makes the new *repleta* an object of great beauty as contrasted with the wild species.

The new fly in all probability came from heterozygous stock, as is shown by the following facts. The original stock was obtained by exposing a fruit jar with banana in a fruit store in North Manchester, Indiana, September 10, 1913. From this bottle[1] there hatched 777 ♀♀ and 206 ♂♂ of *Drosophila ampelophila.* On November 5 appeared *repletas.* November 15, I found one scarlet female among 35 *repletas.* November 16, one scarlet male among 20 flies. November 17, one scarlet female among 25 flies. Some of the virgin flies were isolated and four scarlets appeared on January 24. My assistant, Mr. Powell, also isolated some of the original stock and later found three scarlets. This would seem to show that the stock had mutated some time before being taken into captivity. During September, 1915, I set a great many traps in the region where the above stock was taken,

[1] I should call attention to the aberrant sex ratio found here in *Drosophila ampelophila.* Culture from this stock later gave 491 ♀♀ and 45 ♂♂. I have data on the sex-ratio in this species for over three years and in many different stocks. With this exception I have found it approaching equality. I mated 25 pairs of virgin flies from this stock with the expectation of finding a sex-linked lethal but in each of the twenty-five bottles the sex-ratio was practically one of equality. The subsequent history of the stock was not followed, owing to an accident.

with the hope of finding whether or not scarlet was common in this region. I have bred many of the stocks since that time, but so far no scarlets have appeared.[2]

BEHAVIOR OF SCARLET IN HEREDITY

One of the original virgin scarlet females was mated to a scarlet male. The union was fruitful and a pure scarlet race was produced which has bred true since that time. The sexes are easily distinguished, the life cycle is about thirty days, and after long experience I have found it comparatively easy to breed this fly in captivity.

Scarlet was crossed to a wild stock which had been taken about four months previously in Terre Haute. This stock bred true to black eyes. The flies were studied in mass culture and virgin flies were used in crossing (the sexes were separated every 18 hours). The offspring, which had eyes like the wild stock, were mated in mass culture for the F_2 generation. The following tables give the results from the crosses.

TABLE I

SHOWING THE RESULT IN THE F_2 GENERATION OF CROSSING SCARLET ♀ × WILD ♂

No.	Scarlet ♂♂	Scarlet ♀♀	Black ♂♂	Black ♀♀	Total ♂♂	Total ♀♀	Total Scarlet	Total Black
1	57	64	139	159	196	223	121	298
2	42	27	126	85	168	112	69	211
3	73	31	148	132	221	163	104	280
4	61	61	210	166	271	227	122	376
5	72	73	263	193	335	266	145	456
6	—	—	—	—	—	—	175	530
7	—	—	—	—	—	—	52	182
Total	305	256	886	735	1,191	991	788	2,333

These tables bring out the fact that the new eye color is a simple Mendelian recessive character since it approximates the

[2] It is only fair to state that I had made earlier attempts to find mutations in this species. In the fall of 1911 a female of *D. repleta* was taken in the Zoological laboratory at Columbia University and from this a stock was obtained which was kept going on well-ripened bananas with more or less difficulty for more than a year. It was comparatively easy to keep the colony going in the same bottle by adding food from time to time but difficulty was experienced in founding new colonies. During the period of observation I examined many hundreds of *repletas* without finding a single mutation.

TABLE II

SHOWING THE RESULT IN THE F_2 GENERATION OF CROSSING SCARLET ♂ × WILD ♀

No.	Scarlet ♂♂	Scarlet ♀♀	Black ♂♂	Black ♀♀	Total ♂♂	Total ♀♀	Total Scarlet	Total Black
8	34	37	111	118	145	155	71	229
9	31	56	134	165	165	221	87	299
10	38	46	121	137	159	183	84	258
11	—	—	—	—	—	—	102	300
12	69	91	216	257	285	348	160	473
13	22	19	39	61	61	80	41	141
14	—	—	—	—	—	—	80	264
15	—	—	—	—	—	—	74	230
Total	194	249	621	738	815	987	699	2,194

expected ratio of three to one. There appeared in the F_2 generation from the scarlet male a total of 699 scarlets and 2,194 blacks,—a ratio of 3.14 black to one scarlet. From the scarlet female there appeared in the F_2 generation 788 scarlets and 2,333 blacks,—a ratio of 2.96 black to one scarlet. It is to be noted that the sex ratio is practically one of equality.

ROSCOE R. HYDE

A WING MUTATION IN A NEW SPECIES OF DROSOPHILA

A NEW wing mutation which appeared in my cultures of *Drosophila confusa* Auct. (not Staeg.) is characterized by the fact that the wings curve upward at an angle of about 45 degrees from the region of the tip of the abdomen. The new wing resembles somewhat the shape of a petal of the rose and is easily distinguished from the wild species since the wings of the wild fly project horizontally over and beyond the abdomen, as is characteristic of the diptera. I shall refer to the new fly as jaunty C.[1]

The wild stock from which jaunty C arose was taken in an orchard on the Coss farm about seven miles south of North Manchester, Indiana, in September, 1913. The original stock was bred in a glass vial to which fresh banana was added from time to time. Several stock bottles were made up from this

[1] The wing is like that of jaunty in *D. ampelophila* and is here designated jaunty C(= *confusa*) to call attention to this resemblance.

bottle. All the offspring were examined with a hand lens but no unusual forms appeared until the fourth or fifth generation when jaunty C was discovered. Subsequently three or four similar mutants were found in the cultures, which would seem to indicate that they arose from heterozygous stock. Pure stock was obtained by crossing to the wild flies and "extracting."

When jaunty C is crossed to the wild type all of the flies of the F_1 generation have long wings. No exact record was kept but this statement is true of several hundred that were observed. The sex ratio was practically one of equality. In the F_2 generation jaunty C reappeared, as shown in the following tables.

F_2 GENERATION FROM JAUNTY C ♂ — TABLE I; F_2 GENERATION FROM JAUNTY C ♀ — TABLE II

No.	Jaunty C	Long	No.	Jaunty C	Long
1	40	176	3	37	124
2	38	150	4	24	145
			5	66	308
Total...	78	326		127	577

Among the grandchildren from the jaunty C male the ratio is one jaunty C to 4.18 long, while among the grandchildren of the reciprocal cross the ratio is one jaunty C to 4.54 long. The sex ratios were near equality.

These ratios do not conform very closely to Mendelian expectations, but I have found this species very hard to breed, and since the flies were bred in mass cultures it may be that jaunty C was unfavorably affected by crowding of the larvæ.

I had hoped to carry out more elaborate experiments during the summer of 1914 and had about twenty bottles of the new stock in pure culture and also some wild stocks, when the flies commenced to die during the hot days in the latter part of May and June. Finally the last individual disappeared despite all the care that I could exercise, and no larvæ were left in the bottles to take their place. As the June temperature increased other stocks failed to reproduce and died out. That the warm weather was in all probability responsible is shown by the results which were obtained by placing the stocks in a refrigerator. All those stocks placed in the refrigerator remained very active and continued to reproduce while all the stocks left on the outside died out with the exception of the wild stocks of *D. ampelophila*.

But even *ampelophila* does not thrive when the temperature reaches 100°.

During September, 1914, I took several wild stocks of *confusa* from the same region, and have examined many of the offspring with the hopes of again finding this form but so far no unusual forms have appeared.

Roscoe R. Hyde

MUTATIONS IN TWO SPECIES OF DROSOPHILA

In our cultures of *Drosophila*, mutations have appeared recently in two species other than *Drosophila ampelophila*. Both mutants are characterized by abnormalities in wing venation. One of them has irregular extra veins in the axillary cell, and hence may be called *axillary*. The other is distinguished most clearly by the fusion of the distal end of the second vein to the costa, producing a double vein for a considerable distance, for which reason it is called *confluent*. In each of these cases other abnormal characters are associated with those mentioned, but they are relatively inconspicuous.

The mutant called axillary arose in normal stock of *D. tripunctata* Loew, which has been bred in the laboratory for about six generations. This stock was kept in milk bottles and fed on banana, but received no artificial treatment except anesthesia with ether once per generation. Axillary behaves as a simple Mendelian recessive when crossed with normal, and breeds true in pure cultures.

The mutant called confluent appeared in a culture of an undescribed species of *Drosophila,* referred to as "species B" by one of us in a paper describing its chromosomes.[1] Confluent is a dominant character (*i. e.,* it appears in the heterozygous fly), and so far as we have been able to ascertain it never occurs in the homozygous condition. At least no flies homozygous for it have as yet been found, although numerous matings have been made which should have produced them. The original fly showing the confluent character (a male) appeared in a stock culture, all of his brothers and sisters being normal. He was heterozygous, as shown by matings with normal females, which gave 15 normal and 13 confluent offspring. Seven of the latter, bred

[1] "Chromosome Studies in the Diptera," I, *Jour. Exp. Zool.*, XVII. p. 45, 1914.

to normals in pairs, gave 778 normals and 691 confluents, showing that they too were heterozygous.[2] The remaining six were bred together in pairs and gave 261 normal and 431 confluent progeny, or a ratio of approximately 1:2 instead of the expected 1:3. According to expectation one third of the 431 confluent offspring in this generation should be homozygous, and random matings in pairs (confluent by confluent), should give in five cases out of nine only confluent progeny. Sixteen such matings have been made, none of which gave this result; instead each gave approximately one normal to two confluent, just as did the F_1 heterozygotes. Normal brothers and sisters of confluent in both generations bred *en masse* gave only normals, showing that none of them was heterozygous for confluent. From these data we conclude that the homozygous confluent flies are not viable, and that the 1:2 ratio is due to the total absence of this class. To our knowledge such a condition as this has been previously recorded in only three cases: the "aurea" *Antirrhinum* of Baur, the yellow mouse of Cuenot, Castle, etc., and the dwarf wheat of Vilmorin. Baur's case differs somewhat from the others and from ours in that the homozygous mutant class appears, but soon dies (due to the absence of chlorophyll).

With regard to the origin of mutations the present cases are instructive in showing that they may appear without the use of artificial chemical or physical agents, and without hybridization. No radium, X-rays or any chemicals whatever have been applied to these cultures, except ether, and that only for anesthesia of the adult flies in each generation. The stock of *D. tripunctata* from which axillary arose was obtained wild, and had been inbred for six or seven generations; that of the other species, from which confluent arose, is all descended from one pair of wild flies, almost certainly brother and sister, and had been inbred for about twelve generations when the mutant appeared. In neither case had flies from two localities been crossed; both stocks were pure and inbred. The only agent that could possibly fall under suspicion as a causative one, then, is ether, but this was used uniformly throughout the experiments, and since only two mutations appeared among many thousands of flies, there is no reason for attributing them to the specific

[2] The offspring per pair were respectively: 242 : 194, 93 : 73, 97 : 106, 42 : 47, 133 : 125, 76 : 65, 110 : 94.

effect of ether;[3] a conclusion made even more certain by the fact that other species were bred during the same time, under identical conditions, and with the same treatment, but without the production of mutations. There is every reason to believe, therefore, that the cause of the mutation in each case was purely fortuitous.

One of the aims of our work on the Drosophilas is to apply the chromosome hypothesis to species having chromosomes different from those of *D. ampelophila*. The experimental work of Morgan and others on *D. ampelophila* has pointed directly to the conclusion that the four groups of linked factors which they have studied are located, respectively, in the four pairs of chromosomes of this species. One of us has recently shown in the paper above cited that several other species of *Drosophila* have chromosome groups differing from that of *ampelophila* in the number and relative sizes of the chromosomes. Of the two species considered in the present paper, one, "species B," has six pairs of chromosomes, and should therefore, on the chromosome hypothesis, give six series of linked characters. The other, *D. tripunctata,* has four pairs of chromosomes, but of a type essentially different from that of *ampelophila,* and consequently should also give essentially different linkage series.

It is significant that both of the mutations which we have found (axillary and confluent), are represented by similar mutations in *D. ampelophila*. Judging from these it is not too much to expect that among other mutations which may subsequently arise in our species, some will likewise correspond to some of those in *ampelophila,* and that upon this basis it may be possible to homologize linkage groups, and thus more definitely homologize chromosomes in different species.

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A SEX-LINKED CHARACTER IN DROSOPHILA REPLETA

Drosophila repleta Wollaston (*D. punctulata* Loew) is a cosmopolitan species, though only recently introduced into the

[3] Professor Morgan has arrived at the same conclusion with regard to the appearance of mutations in *Drosophila ampelophila*. Cf. Amer. Nat., 1914, "The Failure of Ether to Produce Mutations in *Drosophila*."

greater part of this country. The color of the thorax (dorsal side), in most specimens, is light gray, each hair having a dark blackish brown spot at its base. These spots are somewhat irregular, and coalesce in certain regions.

In October, 1914, I collected a number of specimens of *D. repleta* in the zoological laboratory at Columbia University. About one sixth of these had a lighter color on the thorax than that found in normal flies. The dark spots, while of about the same number and color as usual, were much smaller and only coalesced in a few small regions. Several females of both kinds were isolated and their offspring observed. These females were, in each case, mated with males of their own kind: but they were of unknown age when captured, and several of them had probably already mated with other males. In the tables given here "dark" refers to the normal type; "light," to the new character.

TABLE I

WILD FEMALES

Culture	Mother	Offspring			
		Dark ♀	Dark ♂	Light ♀	Light ♂
J	Light	5	0	91	86
Q	Light	12	0	50	53
T	Dark	62	76	0	0
U	Dark	71	52	11	41
V	Dark	96	50	0	41
W	Dark	36	30	0	0
X	Dark	32	47	0	0

Light offspring from *J* and from *Q*, when mated together, gave 166 lights in the next generation—no darks. Darks from *T*, mated together, gave 180 darks—no lights.

On the basis of these results it is probable that the light character is a sex-linked recessive. The two light females, *J* and *Q*, had paired with dark males before being captured, since they produced a total of 17 dark offspring: but these darks were all females, showing either that the male-producing sperm of the father carried no dark factor (*i. e.*, that the factor is sex-linked), or that the light character is dominant in the males and recessive in the females.

Female *V*, since she produced light sons but no light daughters, must, on either of the above views, have been mated only by a dark male, and she must have been heterozygous for the light

character. Female *U* must have had the same constitution, but had probably mated with both kinds of males.

The crucial test between the two views was furnished by mating a dark female from culture *T* to a light male from *J*. The result was 25 dark females and 26 dark males. This is the expectation if the character is sex-linked; but if light is recessive in the females and dominant in the males, the mating should have given only dark females and *light* males. The light character is, therefore, sex-linked and recessive.

A further test was made by mating heterozygous females (one from *Q* and one from *U*) by their light brothers. Table II shows that the result approximates to the expected 1:1:1:1 ratio.

TABLE II

Culture	Dark ♀	Dark ♂	Light ♀	Light ♂
*Q*2	12	12	19	15
*U*1	15	17	17	13
	27	29	36	28

In all the cultures it has been observed that the heterozygous females average a little lighter in color than do the homozygous darks. This difference, however, is not sufficient to allow an accurate separation of the two classes. Dark males are of the same color as the homozygous dark females.

In October, 1914, I received some banana collected by Mr. B. Schwartz at Fayetteville, Ark. From it there hatched one *repleta* male, which was of the light type. Bred to light females from culture *J*, this male produced 133 offspring, all of which were light.

An examination of the pinned material in my own collection and that of the American Museum of Natural History has shown the existence of a number of specimens which seem to belong to the light type. The following table shows the distribution of the specimens examined. Those marked "not workable," are not in good enough condition to be classified with certainty.

The table shows the light form to occur in New York, Alabama, Arkansas, California, and Cuba. The Cuban record is of interest because the date, 1904, is the earliest of the seven cases.

TABLE III

Locality	Date	Dark	Light	Not Workable
Woods Hole, Mass.	June, 1913	3	0	
New York, N. Y.	Feb., 1913	2	1	
——	June, 1913	3	1	1
——	Oct., 1914	83%	17%	
Washington, D. C.	Oct., 1912	3	0	
N. Manchester, Ind.	Sept., 1913	2	0	
Daytona, Fla.	Mar., 1914	1	0	
Kushla, Ala.	June, 1914	3	1	
Fayetteville, Ark.	Oct., 1914	0	1	
Claremont, Calif.	May, 1914	4	0	
Newport, Calif.	Sept., 1913	2	6	1
Berkeley, Calif.	1914	2	0	
Near Havana, Cuba	Nov., 1904	3	3	3
Guantanamo, Cuba	Dec., 1913	5	0	1
Roseau, Dominica	June, 1911	5	0	1

At that time *D. repleta* seems to have been rather rare in the United States.

A. H. STURTEVANT

COLUMBIA UNIVERSITY,
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ORIGIN OF SINGLE CHARACTERS AS OBSERVED IN FOSSIL AND LIVING ANIMALS AND PLANTS[1]

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In the last thirty years two biologies have been developing. The first is the biology of the garden, the seed pan, the incubator, and the breeding pen. The second is the biology of the field zoologist, of the field botanist, of the paleontologist. Inasmuch as one regards unnatural processes and the other regards natural processes it is small wonder that these biologies have become as far apart as two religions and have developed their sects and their dogmatists. Yet the actual *facts* assembled in these two biologies as distinguished from the *opinions* based thereupon can not be in the least discordant, for certainly there is only one system of law operating in the living world and there can be only one ultimate and final biology. In my Harvey lecture of 1912[2] the search for some unity between the observations in these two great fields of natural and experimental research met with some failure

[1] Presidential address before The Paleontological Society of America, delivered in the Academy of Natural Sciences of Philadelphia, Wednesday, December 31, 1914.

[2] The present address, as a comparison of zoological, paleontological, and experimental results, is a sequel to the author's Harvey Lecture of 1912, entitled "The Continuous Origin of Certain Unit Characters as Observed by a Paleontologist." Harvey Soc. Vol., 7th ser., Nov., 1912, pp. 153–204. It employs in part the same materials and illustrations.

and some success, and in the present address I am pushing inquiry along the same line, choosing the "single character" as the point of investigation and comparison.

THE ORIGIN OF CHARACTERS

The old and ever vague problem of the origin of species is being resolved into the newer and more definite problem of the origin of characters; in the dim future when we know how and why new characters originate, and how and why they transform and disappear, the problem of natural and experimental research met with some failure and some success, and in the present address I am pushing inquiry along the same line, choosing the "single character" as the point of investigation and comparison.

THE ORIGIN OF CHARACTERS

The old and ever vague problem of the origin of species is being resolved into the newer and more definite problem of the origin of characters; in the dim future when we know how and why new characters originate, and how and why they transform and disappear, the problem of species will have long been solved and well-nigh forgotten. This is because a species is an assemblage or colony of similar individuals, each individual is composed of a vast number of somewhat similar new or old characters, each character has its independent and separate history, each character is in a certain stage of evolution, each character is correlated with the other characters of the individual.

Thus in a sense the species, the subspecies, the variety, even the individual is not a zoological unit, whereas the "character" when narrowed down to the last point of divisibility seems to be a unit both among plants and animals, and a very stable one, with certain distinctive powers, properties, and qualities of its own. We have been approaching this new conception from many different lines of observation among fossil and living animals and plants, and a preliminary survey of results is opportune.

My chief purpose in this address is to show what one of these "single" or "least characters"[3] is and what peculiar powers and properties it possesses which distinguish it from other "least characters" and give it a certain individuality and separateness.

If you read your Lamarck, your Darwin, your Cope afresh with this general conception in mind you will find that throughout biological literature the problem of species has always been an incidental one, a sort of by-problem and relic of the very ancient controversy as to whether species were created suddenly or evolved gradually. The real problem has always been that of the origin and development of characters. Since the "Origin of Species" appeared the terms variation and variability have always referred to single characters; if a species is said to be variable we mean that a considerable number of the single characters or groups of characters of which it is composed are variable. In botany the long overlooked discovery of Gregor Mendel in 1865 had as its most essential feature the separability of characters in heredity. In paleontology as long ago as 1869 Waagen sharply focused our attention on single phyletic characters as of far greater significance and importance than the matter of local races, varieties, and subspecies. The modern observers in experimental zoology and heredity are far less concerned with "species" than with the separate characters of which the individuals within a species are composed.

Some naturalists incline to regard the "character" as observable only by certain methods of their own, but it is obvious that since all hereditary "characters" are germinal there can be no royal or exclusive road by which we may observe their origin and transformation, for the germinal and somatic laws controlling the characters of

[3] T. H. Morgan has pointed out that the term "unit character" was improperly used in my Harvey address. "Unit character" is a germinal rather than a bodily term. I am treating here of single bodily or somatic characters which may be represented by one or more "unit characters" in the germ.

the bean,[4] the fly,[5] the mollusc,[6] the titanothere,[7] and man[8] are doubtless identical.

Accordingly my second purpose in this address is to show that there is a certain harmony in the results obtained in widely different fields of research although some of these results may appear at first to be entirely unrelated and even discordant.

In this attempt to discover an underlying harmony let us first glance at the "character" conception in the older natural sciences of animals and plants. CHARACTER is the most frequently used term in the vocabulary of zoology and botany. It occurs far more often than any other word. It has been used millions of times in systematic definition since Linnæus. Yet I do not know of any attempt to clearly define or analyze the meaning of the word character in its biologic sense while hundreds of attempts have been made to define the word species. Here again the greater is involved in the less and whenever we shall succeed in clearly defining "character" the definition of species will follow as an incidental result.

The derivation of the word is from the Greek χἄρακτήρ properly an instrument for marking or graving; as applied to a person, an engraver; as commonly used, any mark engraved or impressed, the impress or stamp on coins and seals. It passes into the word characteristic, which means a distinguishing feature.

The use of the word is not only universal among systematists and experimentalists of our day, but it has become one of the most elastic words in our language; the "character" may be as comprehensive as the general habit of an entire organism, as where we speak of the lethargic character of the sloth, or as restricted as a single minute cuspule on a fossil tooth, or the barely visible outgrowth on the surface of a fossil shell. The speed of the race horse is a character, its tractability or viciousness are characters, the position of the horse's tail in running

4 Johannsen.
5 Morgan.
6 Waagen, Neumayr, Hyatt, Jackson.
7 Osborn.
8 Galton.

is a character, the color of the horse's hair is a character, the most minute cellular structure of the tissue of the hoof is a character.

There is an underlying reason why this very elastic use of the term is absolutely scientific: it is, that every one of the above diverse applications of the term to animal or plant life refers to some structure or some quality which is *heritable;* heredity is the unifying principle.

The word is again elastic and often confusing in being used both for germinal characters which are always heritable and for bodily modifications of character acquired through habit or environment which may not be heritable. When we speak of characters which are not known to be hereditary we should qualify them as acquired, as modified, as due to nurture, to habit or ontogeny, to environment, as somatic rather than as germinal. Thus it is perfectly proper to speak of "ontogenetic species" as Jordan does, species the bodily characters of which are due to certain habits; or of "environmental species" the bodily characters of which are due to peculiarities of environment. While such modifications by habit and by environment make up a considerable part of the characters which distinguish geographic species, subspecies and races, it is not the origin and the transformation of these characters which we are now considering, for that problem is comparatively simple, but rather of those underlying *germinal* and *heritable characters* the origin and transformation of which is absolutely an impenetrable mystery at the present time.

How do we know through zoology, botany, and paleontology as well as through experiment that "characters" are real units of structure with some individual and distinct qualities and properties of their own which separate them from all their fellows and at the same time with certain properties of correlation which unite them with all their fellows?

First, we may observe in these living and extinct forms evidences of two such antithetic principles, a principle

of *hereditary separability* whereby the body is a colony, a mosaic of single individual and separable characters, which is combined with a principle of *hereditary correlation* whereby the body is a complex of minutely related and interacting units so that functionally and structurally many of these units are linked with others. Neither principle is simple; on the contrary, both principles are extraordinarily complex and go back to the very beginning of things. Comparing more closely the observations on fossil vertebrates and invertebrates, we develop *laws of separability* as well as *laws of correlation*, and note that certain of these laws are far more clearly perceived in some fields of observation than in others.

The biologic value of the field to which our Paleontological Society is especially devoted lies in the revelation of certain of these laws and causes of the separability of characters which are not revealed at all to the zoologist or to the experimentalist. The paleontologist is in a position to understand *why* certain characters fall apart and become separable in cross breeding, the cause being connected with their origin and antecedent history.

Of far broader biologic significance is the fact that all principles which may be discovered through paleontology regarding the "origin of characters" in the hard parts, govern alike characters of the soft parts as well as of other structures and functions. For there can not be one principle governing the "characters" of bones, another those of the muscles, another those of nerves; one principle for structures, another for functions. But while these principles are unlimited, our *comparisons* with zoology, for example, are limited to the origins of characters which may be observed both in living and fossil forms, namely, in the skeleton and in the teeth; and at the outset a convenient and readily understood distinction may be made between the origins of *numerical* and of *proportional* characters, as follows:

Numerical	*Proportional*
Presence and absence characters, *e. g.*, numbers of teeth, of cusps on the teeth, of vertebræ, of toes, of pads on the feet, of mammæ. Meristic or segmental characters, such as may be partly expressed in *formulæ*.	Changes of form in the length, breadth and height of parts. Quantitative changes in the hard parts. Such characters as may partly be expressed in *indices* and *ratios*.

Proportional characters may through prolonged reduction lead into numerical characters. Thus the reduction in length of one of the toes may precede the loss of the toe, which is a numerical change. Yet we shall see that somewhat different principles prevail in the origins of certain numerical characters as contrasted with the origins of proportional characters.

1. *Use of Numerical and Proportional "Characters" in Classification of Mammals*

In our attempt to analyze "characters" as they are revealed to the systematic and field zoologist let us take as two examples, first, "The Catalogue of the Mammals of Western Europe" by Gerrit S. Miller,[9] and, second, the "Revision of the Mice of the genus *Peromyscus*," by Wilfred H. Osgood. It is of the utmost importance that mammalogists, whether working among living or fossil forms, should use similar methods of description and definition of characters, and we especially welcome in the monumental work of Miller the fact that the definitions and the keys are chiefly upon the hard parts which are also available to the paleontologist. We select as typical his treatment of the Order Carnivora and of the Family and Genera of the wolves and foxes, which he distinguishes by the following enumeration of characters:

Miller's Diagnoses and Definitions	*Our Analysis of the Kinds of Characters*
ORDER CARNIVORA. *Characters.* —Terrestrial (rarely aquatic or	Chief habits, chief adaptations of the teeth and limbs; chief char-

[9] Miller, Gerrit S., "Catalogue of the Mammals of Western Europe (Europe exclusive of Russia) in the Collection of the British Museum." London, 1912, 1019 pp.

semi-aquatic), non-volant, placental mammals with rather high development of brain. The cerebral hemispheres with distinct convolutions; feet unguiculate, never modified as fins or flippers; dentition of a modified tuberculo-sectorial type, the posterior upper premolar and anterior lower molar usually developed as special carnassial or flesh-cutting teeth.

acters of the brain. Inherited from "least characters" which accumulated and evolved in Mesozoic and early Tertiary time.

Family CANIDÆ. *Characters.*—Larger cheek-teeth of a combined trenchant and crushing type, the last upper premolar and first lower molar strongly differentiated as carnassials, the former 3-rooted, its inner lobe in front of middle of crown, its position, somewhat posterior to level of anteorbital foramen, at point of greatest mechanical efficiency; auditory bulla moderately or considerably inflated, without septum; form rather light, the legs long; size moderate; feet digitigrade; toes, 5–4 or 4–4.

Characters of proportion and changes of form; characters of function or adaptation; presence or absence of certain numerical characters. Characters by which the ancestors of the family diverged as terrestrial and cursorial Carnivora from other Carnivora.

Genus CANIS. *Characters.*—Skull heavy and deep (depth of brain-case more than one-third condylobasal length); interorbital region thickened and elevated, the frontal sinuses rather large, the postorbital processes thick, convex above, their edges rounded off; dorsal profile of forehead rising rather abruptly and noticeably above level of rostrum; dental formula;

$$i\frac{3-3}{3-3},\ c\frac{1-1}{1-1},\ pm\frac{4-4}{4-4},\ m\frac{2-2}{3-3}=42;$$

teeth heavy and large, the length of carnassial and upper molars together contained about 2½ times

Chiefly characters of proportion; also numerical characters of the teeth. Characters clearly manifested in lower and middle Eocene time and taking on their modern aspect in early Oligocene time.

in palatal length; canines robust and not specially elongated, the point of upper tooth extending scarcely beyond middle of mandibular ramus when jaws are closed (Fig. 65).

[Species] CANIS LUPUS. *Diagnosis.*—Condylobasal length of skull more than 200 mm. (220 to 225 mm.); cheek-teeth larger than in the largest races of domestic dogs, the upper carnassial 25 to 27 mm. in length, but structure not peculiar, the upper molars with narrow, inconspicuous cingulum on outer side (Fig. 61).

Chiefly characters of proportion; certain minor numerical characters. Characters distinguishing *Canis lupus* from *Vulpes,* first apparent in Miocene and Pliocene time.

[Subspecies] CANIS LUPUS LUPUS. *Characters.*—Size maximum for the species; general colour not markedly tawny; white of throat not extending to cheeks. The few skulls examined agree with Asiatic specimens in having the outer cusps of m^1 moderately large, the paracone with transverse diameter of base about equal to width of large flattened portion of crown.

Size characters; color characters; proportions of dental cusp characters. Characters fully developed in early Pleistocene time, perhaps 500,000 years ago.

In the ascending order of Miller's definitions we note that "subspecies are mainly distinguished by characters of proportion and of form and by the degrees and intensities of color, but rarely if ever by numerical characters. "Species" are mainly distinguished by the proportions of the various hard parts and to a less extent by the presence and absence of minor "numerical" characters. "Genera" are distinguished by the proportions, by the presence or absence of several numerical characters, also by functional characters such as dental succession. "Families" are distinguished by changes of proportion and of form, by many numerical characters, such as the presence or absence of certain parts, by structural adaptations in the teeth and feet. "Orders" are distinguished by the funda-

mental and very ancient chief habits, chief adaptations in the hard parts, chief brain features.

Thus we see that two kinds of characters are employed by Miller throughout, namely: first, characters of proportion of form and of degree; second, numerical or

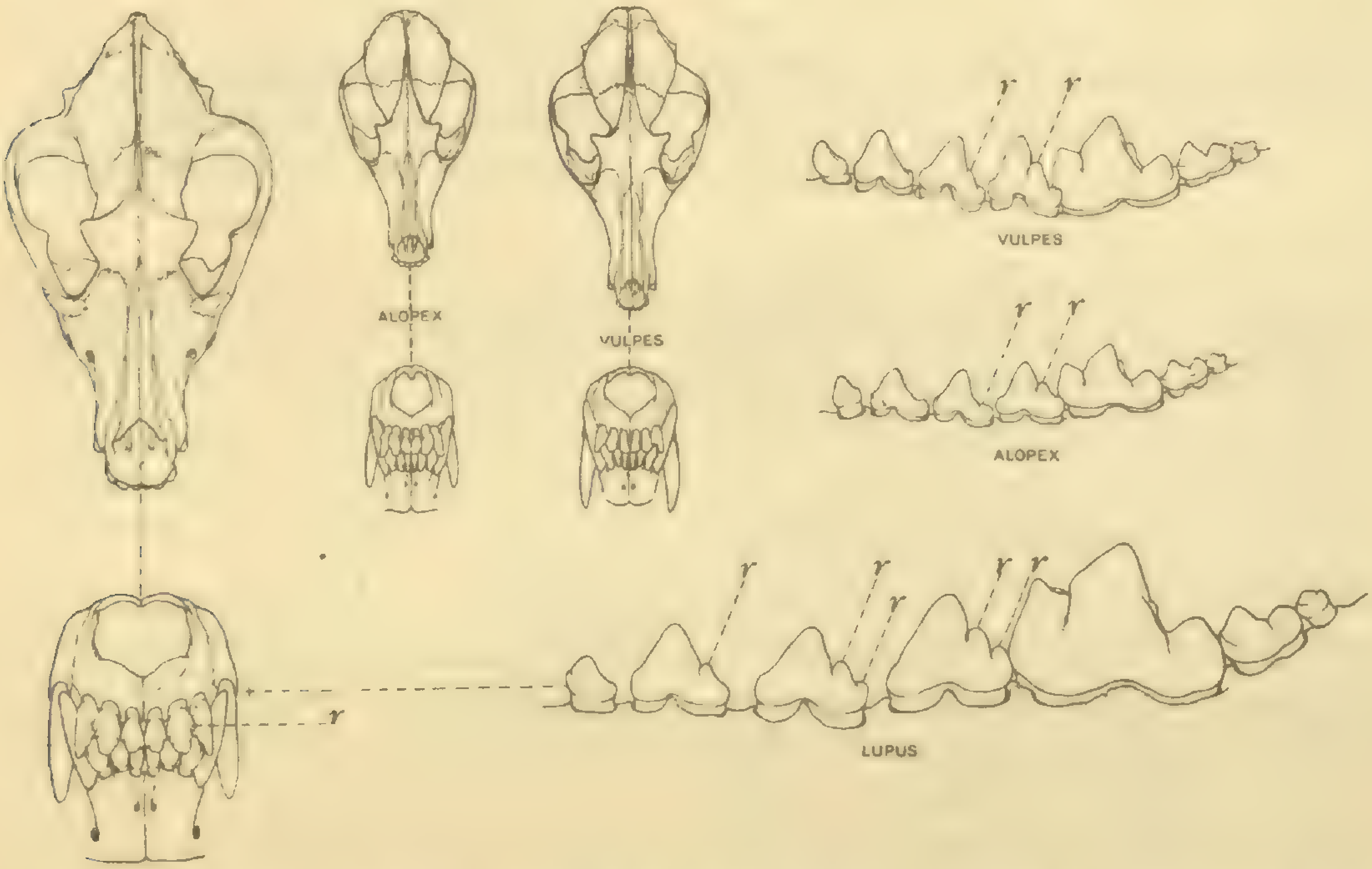

FIG. 1. Skulls and cheek teeth of the wolf (*Lupus*), arctic fox (*Alopex*), and red fox (*Vulpes*), illustrating the differences in proportional characters of the skulls and teeth and the resemblances in the numerical characters, or rectigradations (*R*).

presence and absence characters. We are struck by the fact that changes in proportion embrace by far the larger part, perhaps nine tenths, of the "characters" enumerated by Miller in his systematic descriptions; this is because *change of proportion is the chief and most universal phenomenon in the adaptation of mammals to different habits and habitats. Numerical change is hardly less important, but is less universal and less frequent.*

Similar weight upon the value of characters of proportion is seen in the contrast between Miller's definitions of the three genera of dogs, namely: *Canis, Alopex,* and *Vulpes.* Here again the vast majority are characters of proportion and of form.

I. Genus CANIS

Characters. — Skull heavy and deep (depth of brain-case more than one-third condylobasal length); interorbital region thickened and elevated, the frontal sinuses rather large, the postorbital processes thick, convex above, their edges rounded off; dorsal profile of forehead rising rather abruptly and noticeably above level of rostrum; dental formula:

$$i\frac{3-3}{3-3},\ c\frac{1-1}{1-1},\ pm\frac{4-4}{4-4},\ m\frac{2-2}{3-3} = 42;$$

teeth heavy and large, the length of carnassial and upper molars together contained about 2½ times in palatal length; canines robust and not specially elongated, the point of upper tooth extending scarcely beyond middle of mandibular ramus when jaws are closed (Fig. 65).

II. Genus ALOPEX

Characters. — Skull intermediate in general form between that of *Canis* and *Vulpes;* occipital depth about one-third condylobasal length; interorbital region more elevated than in *Vulpes* owing to greater inflation of the frontal sinuses; postorbital processes thin, flat or slightly concave above, with bead-like, overhanging edges; dorsal profile of forehead rising abruptly above rostrum as in *Canis;* teeth moderately heavy and large, the length of carnassial and upper molars together contained about 2¾ times in palatal length; canines and incisors intermediate between those of *Canis* and *Vulpes* (see Fig. 65); external form fox-like, but ear short and rounded, not conspicuously overtopping the surrounding fur.

III. Genus VULPES

Characters.—Skull slender and low (depth of brain-case less than one third condylobasal length); interorbital region nearly flat, the frontal sinuses scarcely inflated, the postorbital processes thin, slightly concave above, their edges overhanging and bead-like; dorsal profile of forehead rising very slightly and gradually above level of rostrum; dental formula as in *Canis;* teeth relatively light and small, the length of upper carnassial and molars together contained about 2¾ to 3 times in palatal length, the general character of cheek-teeth somewhat more trenchant than in *Canis,* the canines slender and elongated, the point of upper tooth extending to about lower margin of mandibular ramus when jaws are closed (Fig. 65).

The fact that changes of proportion include the most frequent characters while numerical changes include the least frequent characters is again very strikingly brought out in Miller's remarks on the origin of the domestic dogs from the wolf (*Canis lupus*):

The only known characters by which the skull of *Canis lupus* can be distinguished from that of the larger domestic dogs is the greater average general size and the relatively larger teeth. In a dog's skull with condylobasal length of 230 mm. the length of upper and lower carnassials is, respectively, 21.6 and 25.0 mm. In ten skulls with condylobasal length of more than 200 mm. the average and extremes for these teeth are: upper, 20.5 (19–22); lower, 24.0 (22.8–26.0). In all the dog skulls which I have examined, representing such different breeds as the pug, fox-terrier, bloodhound, mastiff, ancient Egyptian, ancient Peruvian, Eskimo (Greenland and Alaska) and American Indian, the teeth are strictly of the wolf type, never showing any approach to that of the jackal (Fig. 62).

This indicates that the profound differences of osteological character which separate the larger breeds of domestic dogs are chiefly in the proportions.

No numerical, or presence and absence characters are used in Miller's definition of the wolf, arctic fox, and red fox although a number of minor numerical characters are clearly described and figured in his text, especially the cuspules on the incisor and premolar teeth, as shown in Fig. 1. These numerical cusp characters would have received more attention from a paleontologist partly because of the paucity of material which comes into his hands, partly because he is in a position to observe the development of these cuspules.

This contrast between proportional and numerical characters brings out a fundamental law in the evolution of the hard parts of mammals which is of great importance. First, characters of form and proportion, without numerical change, are constantly originating as a universal principle and forming the chief distinctions between divisions from the high rank of orders down to those of subspecies, races, and even individuals. Second, numerical loss or fusion of old characters of teeth, digits, or vertebræ is next in frequency, the loss always following diminution in form and proportion. Third, numerical gain of new characters is the least frequent process; it is relatively rare in the endoskeleton, that is, in added teeth, added vertebræ and other segmental parts, added cranial bones, added phalanges; it is more frequent in added cusps on

the teeth or added horns and appendages of the skull; it is still more frequent in added exoskeletal characters, such as dermal ossicles and armatures.

The contrast between the wolf (*Canis lupus*), the arctic

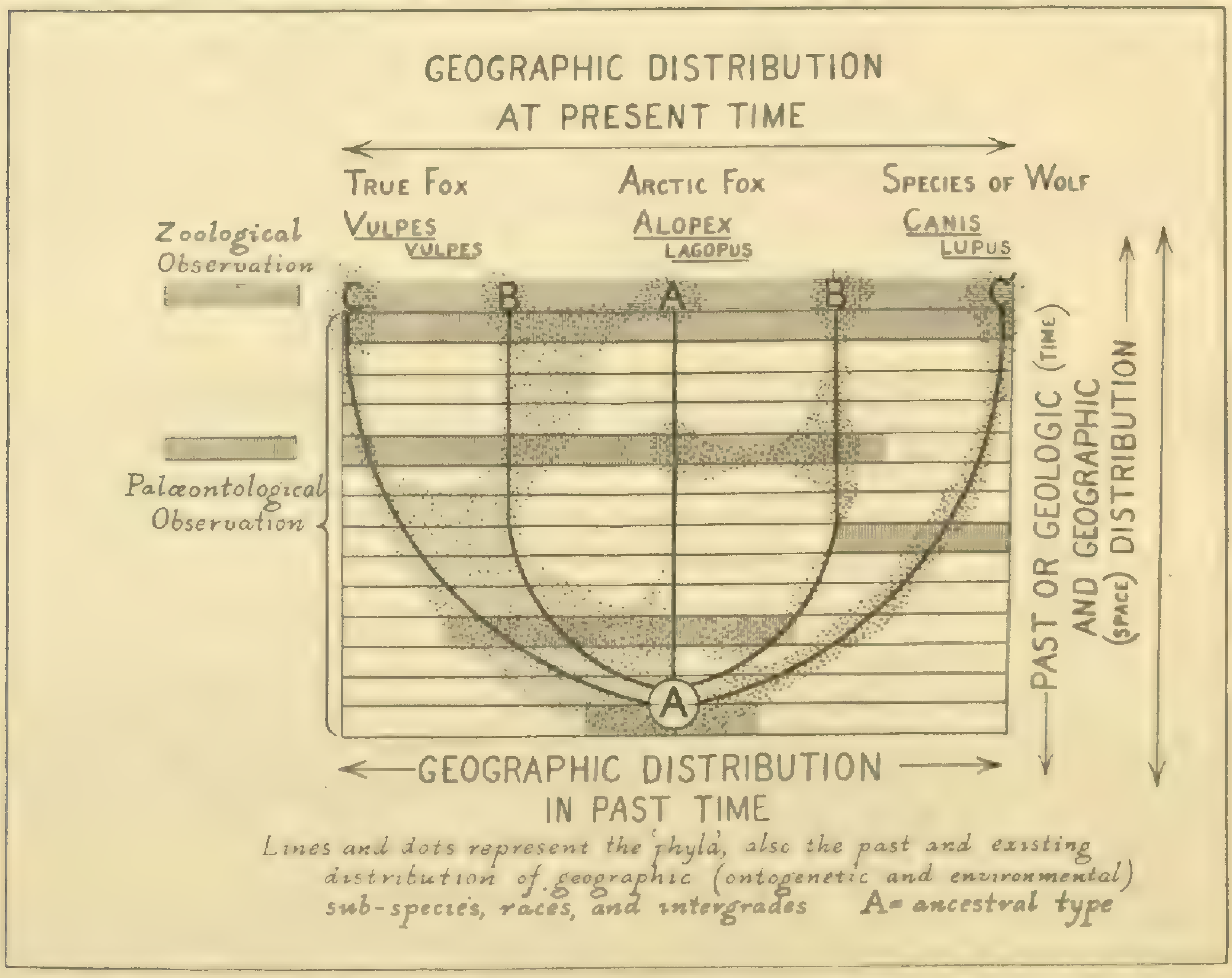

FIG. 2. Diagram illustrating the theoretic descent of the dogs from a common geologic ancestor A. Dots = intergradations observed by the paleontologist (vertical lines) at five different intervals of geologic time. A B C = existing forms with a few existing intergradations (dots) observed by the zoologist (horizontal lines).

fox (*Alopex lagopus*), and the red fox (*Vulpes vulpes*) may, moreover, be adduced for four purposes: first, to direct attention to the nature of the *numerical characters* which separate these three genera; second, to direct attention to the fact that these numerical characters are very inconspicuous and unimportant in contrast with characters of proportion; third, to illustrate the extremely slow development in time of new numerical characters; fourth, to illustrate the difference between paleontological and zoological observation, a difference which is graphically represented in the diagram (Fig. 2).

As to time *Vulpes* has been separated from *Canis* for an

enormous period.[10] It is clearly distinguishable in the European Pliocene where three species of canids are referred to the genus *Vulpes* by Schlosser and others. Again, in the Upper Pliocene of India there occurs a species of fox as well as in the Pliocene of China. In North America the fox is first recorded from the Pleistocene definitely, although an Upper Miocene species (*Canis vafer* Leidy) is regarded by some as the forerunner of *Vulpes* and by others as a pro-*Vulpes* genus. It is therefore probable that the phylum of the fox diverged from that of the wolf as early as Miocene times, perhaps a million years ago, although the generic distinctions of proportion-characters were not fully acquired until Pliocene times. The ancient geologic separation of *Canis* and *Vulpes* is further indicated by the fact that they do not interbreed. The marked divergence in proportions—the fox small, slender, narrow-headed, a small-mammal and bird catcher, the wolf relatively large, massive, broad-headed, a large-mammal catcher—is accompanied by the gain or loss of several *relatively obscure* numerical characters, such as the cuspules on the incisors and premolars (Fig. 1. *r, r, r*), which are strong in the wolf, intermediate in the arctic fox, and absent in the red fox. It would appear that the wolf had developed these numerical cusp characters more rapidly than its congeners. In a fossil series the development of such cusp characters may be followed stage by stage.

2. *Observations by a Field Zoologist on the Modes of Origin of Numerical and Proportional Characters*

The special features of the field work developed under C. Hart Merriam's direction in the U. S. Biological Survey are: (1) the vast quantity of comparative material brought under examination, (2) the exact geographic, climatic and environmental records, (3) the assemblage of numerous intergradations between species and sub-species, (4) the precision of the measurements and observa-

[10] I am indebted to the authority of Dr. W. D. Matthew for these remarks.

tions, but above all (5) that the *facts are recorded entirely without the influence of any biological theory*, the mind of the observer being absolutely fresh and unprejudiced.

The observations published in 1909 by Wilfred H. Osgood on the mice of the genus *Peromyscus*[11] therefore constitute a notable and wholly unbiased research on bodily "characters" as they appear to a zoologist collecting and observing in the field, but examining and reviewing his material in the museum. The following abstract is mainly in the author's own language and has been verified by him, although the order of treatment is rearranged entirely and italics are added for emphasis in its bearing upon the modes of origin of single characters in living mammals.

As recorded there have been examined more than 27,000 specimens of the American rodent genus *Peromyscus* (Gloger, 1841), including the so-called wood mice, deer mice, vesper mice, or white-footed mice, having a total range from the Mexican province of Oaxaca on the south to the Yukon, Alaska, on the north, and from Labrador to Florida on the east to Alaska and southern California on the west.

The "genus" *Peromyscus* is for convenience divided into five "subgenera," which are distinguished mainly by the presence or absence of three *numerical* characters, namely, tubercles on the soles of the feet five or six, presence or absence of accessory tubercles on the first and second molar teeth, presence or absence of two or three pairs of mammæ. The remaining subgeneric characters lie in *differences of proportion* and in *color relations*. The "subgenera," which are usually defined by a combination of characters, may merely represent *opposite ends of an almost continuous series* (*e. g.*, *Haplomylomys-Peromyscus-Megadontomys*). Intergradation is observed also in certain of the numerical characters, as in the six to vestigial five plantar tubercles of the *P. maniculatus* group.

The species of *Peromyscus maniculatus* (Wagner), alone including forty-four subspecies, ranges from Vera Cruz to Labrador and has a wider distribution and a larger number of intergrading forms than any similar group of mammals known. From the typical *P. maniculatus development may be traced step by step absolutely without break through all the numerous subspecies.*

Perfect integradation, in proportion and color intensity and distribution characters, is observed between the related forms ("sub-

[11] Osgood, Wilfred H., "Revision of the Mice of the American Genus *Peromyscus*," U. S. Dept. of Agric., Bureau of Biol. Surv. No. Amer. Fauna, No. 28. Apr. 17, 1909, 285 pp.

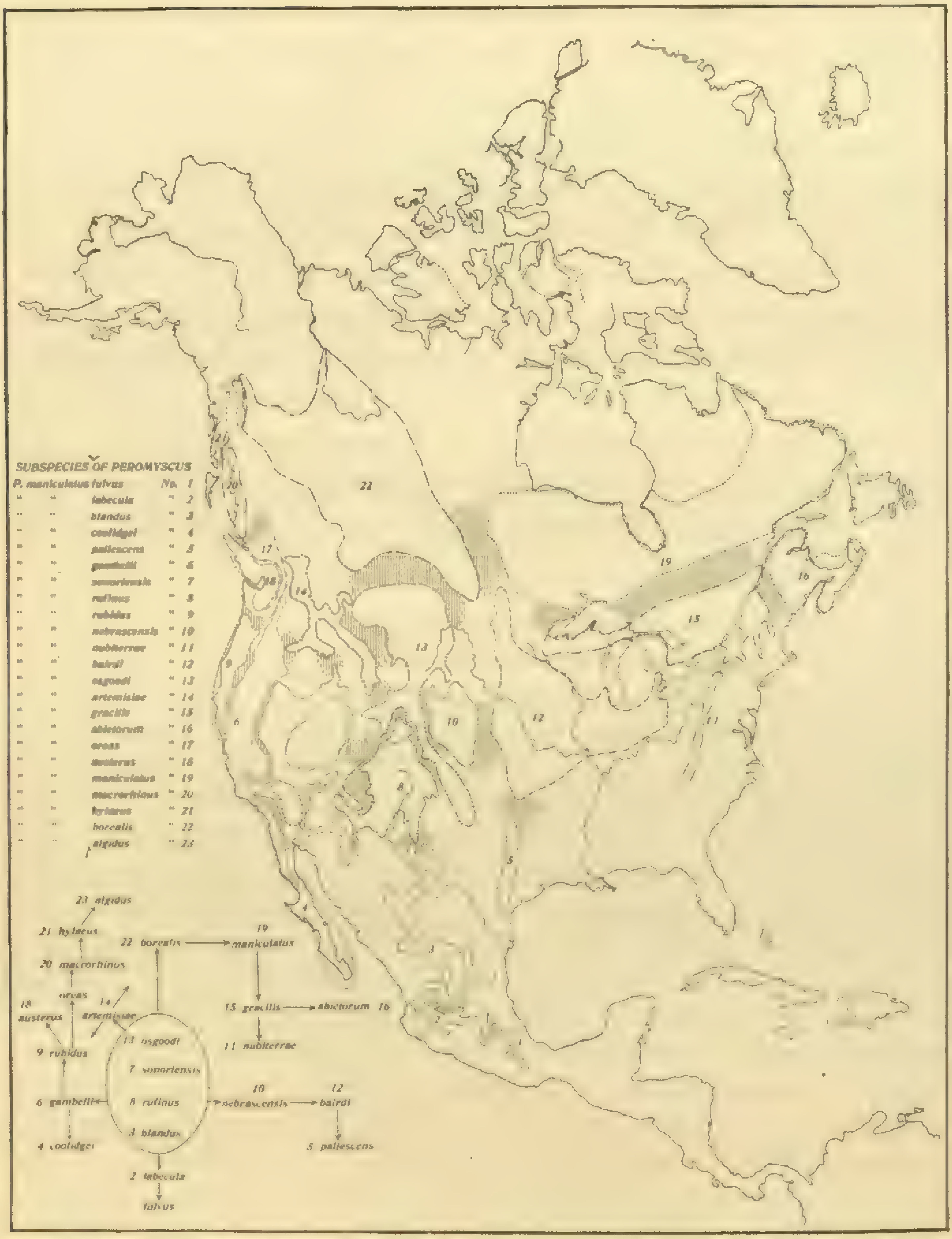

FIG. 3. Geographic distribution of the subspecies of *Peromyscus maniculatus*. The geographic boundaries of each subspecies are represented by continuous or variously dotted lines. The areas of complete intergradation or continuity between two subspecies are indicated by vertical shading. Where there is no shading there is an apparent discontinuity which, as indicated in the diagram in the lower left-hand corner, is not real; this diagram shows the various continuous chains of subspecies and intergradations the terminal members of which appear to be *discontinuous*.

species") of the many different faunal areas. Hundreds and even thousands of specimens are intergrades almost equally resembling two or more adjacent forms. Many specimens fall so near an imaginary line between two or more "subspecies" that it is practically impossible

to classify them other than as intergrades. Particularly difficult cases are those in which the intergrades approximate the color of one "subspecies" and the cranial characters of another, thus reducing the question of definition to one of the relative importance of characters. Classification becomes like the division of a spectrum and depends largely upon the standards set, for theoretically at least the possibilities of subdivisions are unlimited.

Some of the principles of variation [and perhaps of hybridization, H. F. O.] are as follows:

(1) Fortuitous individual variation is greatest in specimens from localities lying just between the ranges of two well-established forms.

(2) Where two genuine "subspecies" inhabit the same area and maintain themselves distinct, each may in certain cases be traced by a definite geographic route through every degree of intergradation to one parent form. For example, *P. arcticus* lives side by side with *P. algidus* in the upper Yukon, but both intergrade toward the south with *P. oreas* (see Fig. 3). If from sudden or gradual natural causes these intergrades between *P. arcticus, P. oreas,* and *P. algidus* were to become extinct three entirely separate and distinct subspecies would apparently be created.

(3) Sexual variation in proportional characters is so slight as to be practically unmeasurable.

(4) The "species" are fairly well characterized in cranial proportions, but the cranial proportions in "subspecies" are seldom constant throughout a series although they often afford average proportional characters of considerable value. For example, among "species" that are normally brachycephalic a greater or less tendency to dolichocephaly is sometimes found, and *vice versa*. The teeth vary chiefly in proportions but seldom to great extent. Some subspecies are dichromatic.

(5) In color there is a range of seasonal, polychromatic, and local or geographic variation. A complete intergradation between two color extremes may often be found in localities lying just between the ranges of two well-established forms. Color intensities are often extremely local and doubtless are produced immediately upon contact with certain environments. Thus if the range of a given subspecies includes a few square miles of lava beds, specimens from that area show appreciably darker color than the normal members of the subspecies occupying the surrounding region. Again, specimens from the bottom of a dark, wooded cañon may be noticeably darker than those inhabiting an open hillside only a few hundred yards away. One can hardly avoid the suspicion, observes Osgood, that if the progeny of paler individuals were transferred at an early age to the habitat of the darker ones they would quite regardless of heredity develop darker color. Such local "geographic variations" are so great that most of the species have developed geographic peculiarities by means of which

they have been subdivided into numerous "geographic races" and "subspecies." Thus *P. maniculatus,* which ranges from the Arctic Circle to the Isthmus of Tehuantepec, remains constant only where the environment is identical, hence it is represented by definable "subspecies" in almost every faunal area which it enters (see Fig. 3).

These observations of Osgood may be compared with the taxonomic results of Miller on the one hand and with the observations of paleontologists on the other:

First: we note that in the "species" of the subgenus *Peromyscus* and in the "subspecies" of *Peromyscus maniculatus* among the vast number of characters enumerated there is not a single distinction recorded in numerical or presence and absence characters; every single character recorded is either in the proportions of the skull, ears, feet, and tail, or in the intensities and distribution of the color areas—all characters of degree. The field and museum work of Osgood thus independently accords with the taxonomic work of Miller, namely, "proportional characters" are universal and abundant, "numerical characters" are less frequent and of a higher or different taxonomic order because much more gradual in evolution.

Second: *the evidence for continuity in the origin of proportional characters is absolute.*

Third: in a broad way *continuity is also the mode of origin of the so-called numerical characters* for it is positively observed except in the case of the mammæ, and there is no apparent reason, remarks Osgood, why the mammæ also may not have developed in the same way as the more trivial characters. In other words, there is almost complete continuity between groups which many taxonomists would regard as different "genera." The numerical differences in the plantar tubercles on the soles of the feet have not been sufficiently studied, but it is clear that the change from 5 to 6, or *vice versa,* has come through the gradual reduction or growth of one tubercle and not through any sudden change. Most interesting also is the fact that the 5-tubercled *Peromyscus* shows decided similarity to the genus *Onychomys,* which is 4-tubercled and closely allied to *Peromyscus.*

Fourth: while the numerical characters are solely germinal, it is difficult or impossible to distinguish both in respect to color intensities and to proportions, what is germinal, permanent and hereditary from what is somatic or due to environmental and ontogenetic influences.

These four chief conclusions drawn from the observations of Osgood may now be compared with those independently obtained by paleontologists.

3. *Likeness and Unlikeness Between Paleontologic and Zoologic Observation*

The mammalian paleontologist observes exactly the same *kinds* and *degrees* of characters as the zoologist, namely, very numerous changes of proportion and form, and relatively infrequent numerical changes. In both respects, however, the paleontologist has the very great advantage of observing the extremes and also many of the intermediate stages.

The chief distinction between these observers is that as the zoologist sees characters they are stationary, he can only infer their separability in movement through his inferences from the comparison of forms like *Canis, Alopex,* and *Vulpes,* while the paleontologist observes several new evolution properties in these same "characters," namely their actual *movement* and their relative *rate of movement* in various lines of descent, as well as their *origin* and subsequent progression or retrogression, in brief, their *phyletic history.* Thus the paleontologist is in a position to observe more of the evolution properties in characters of exactly the same kind. Whereas in a series of living forms each character appears to the zoologist-observer as dead or static, in a fossil series each character appears to the paleontologist-observer as living or dynamic, the life being displayed in what may be called its two *movements in a phyletic series.*

The first property of the *ontogenetic movement* of characters in fossils constituted the life work of our great observer Alpheus Hyatt, who proposed the significant and easily recalled terms *acceleration* and *retardation* for the

two directions of movement seen in ontogeny and phylogeny. Accelerated characters are those which hurry forward and appear in successive generations at earlier and earlier stages in the development of the individual; while retarded characters are those which hold back or slow down and appear in later and still later stages in the development of the individual of succeeding generations. We know that such ontogenetic movement is shown both in embryonic and phylogenic development of the individual; it causes characters to appear in ontogeny out of the order in which they arise in phylogeny; it gives rise to the heterochrony of Gegenbaur; its rate is measured by comparing one character with all the other characters of an individual.

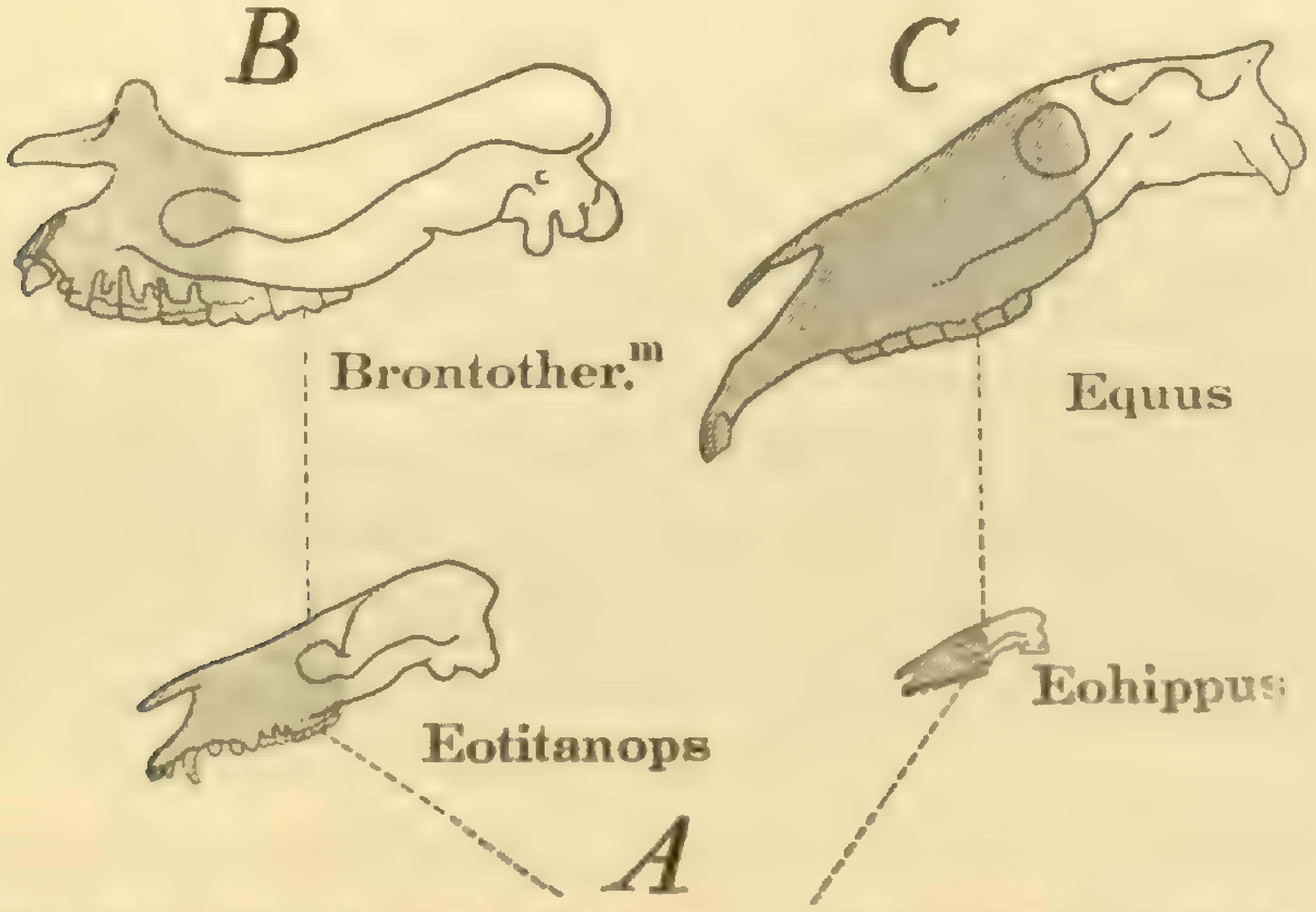

FIG. 4. Evolution of profoundly different proportional characters (*B*, *C*) from ancestors (*A*) having similar proportions.

Quite distinct is what we may call the *phyletic movement* of a character; its rate is measured by comparing a character in individuals of one phylum with the same character in individuals of other phyla. It is illustrated in the comparison of the secondary cusps of the incisor and premolar teeth in *Canis*, *Alopex* and *Vulpes;* in each phylum the same cusp has its distinctive rate of evolution and thus may appear early in geologic time or late in geologic time. Thus comparison of the phyletic movement of the same "character" in various lines of descent,

which is a matter of phylogeny, is quite different from comparison of the relative movement of a number of different characters in single lines of descent, which is the basis of Hyatt's law.

To illustrate the distinction between ontogenetic and phyletic movement: a rudiment of a horn may appear upon the skull in one phylum of titanotheres during the period of deposit of the base of the Bridger beds (Fig. 5), which are 1,500 feet in thickness, and in another phylum (Fig. 5) at the summit of these beds, many thousands of years later; this is its relative *phyletic movement.* Second, after the same horn-character has appeared long subsequent to the birth of the individuals, in both phyla it begins to be thrust forward in the ontogeny of individuals, so that in Lower Oligocene time it begins to appear long before birth; this is its acceleration or *ontogenetic movement.*

Paleontology has also revealed the marked distinction in the mode of origin of the two kinds of characters observed in zoology, namely, between the almost universal changes of proportion and the comparatively rare new "numerical characters."[12]

To the former I have applied the term *allometrons,*[13] which signifies that differences of measurement express all changes of proportion. From these differences indices and ratios may be calculated. Such differences arising in the head and in the feet are indicated in the familiar terms dolichocephaly, brachycephaly, dolichopody, brachypody, and many other convenient combinations of Greek terms. That these changes of proportion become distinct hereditary "characters" is proved in certain hybrids of mammals where they appear to be partly or completely separable. Thus the cross of human broad-heads with long-heads does not produce a blend between the two but produces, for some generations at least, either pure doli-

[12] Osborn, H. F., "Coincident Evolution Through Rectigradations (Third Paper)." *Science*, N. S., Vol. XXVII, No. 697, May 8, 1908, pp. 749–752 (p. 752).

[13] "Biological Conclusions Drawn from the Study of the Titanotheres," *Science*, N. S., No. 856, May 26, 1911, pp. 825–828 (p. 826).

chocephals or pure brachycephals. Characters of proportion are thus "single characters" in the hereditary sense.

In the comparison, for example, of certain broad-heads with other broad-heads such characters are termed *analogous* because due to similarity of structure arising from similarity of function. Thus brachypody (abbreviation of the digits) is analogous in the rhinoceroses and the titanotheres. The broadening of the shell of one mollusc is analogous to the broadening of the shell of another mollusc. The broadening is none the less the heritable characteristic of the skull or of the shell.

Quite different are certain of the new numerical characters to which I have applied the term *rectigradations*,[12] such as new cuspules on the teeth and new rudiments of horns, for these give rise to characters which are regarded as *homologous* although not directly descended from each other. Thus the horns in all the titanotheres are considered homologous, although they arise independently at different times in different phyla. The larger number of cusps in the teeth of mammals are termed homologous, although they also have arisen quite independently of each other. It is obvious that unless all similar new characters have originated in the offspring of a single pair, which we know is not the case, *that the vast majority of similar new numerical characters both in vertebrates and invertebrates are related through similarity of ancestry, through the similarity of the tissues from which they arise, and through the similarity of their relations, forming a special kind of homology which Fürbringer has termed homomorphy.*

While different in these respects of analogy and homology there are many properties which allometrons and rectigradations as heritable characters have in common, such as the laws of growth, correlation with sex, mechanical correlation, differential ontogenetic movement, differential phyletic movement, or differential rates of evolution, continuity of origin, increasing intensity of

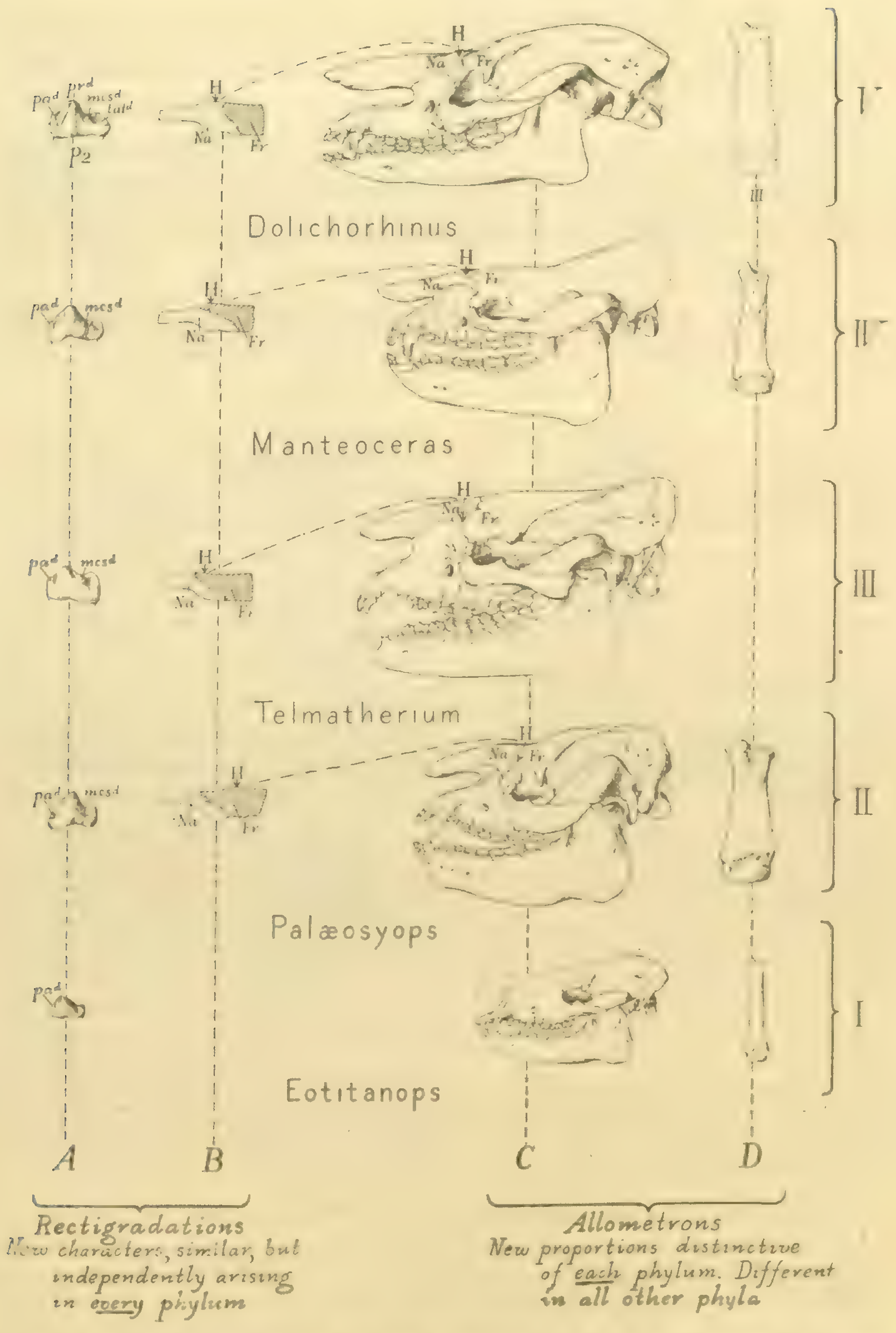

FIG. 5. The independent origin and evolution of similar numerical characters (*A*, *B*) and of dissimilar proportional characters (*C*, *D*) all arising independently in descendants of the same ancestors. Each of the five phyla (I–V) exhibits similar rectigradations (*pad*, *mesd*, *H*) and dissimilar proportions both in the skulls and metapodials.

development in successive generations. For example, a rectigradation like the hypocone may become more distinct, a change of proportion like brachycephaly may become more pronounced in successive generations. Yet there are a number of additional contrasts between the proportional and certain numerical characters, a few of which may be enumerated:

Proportional characters = *Allometrons*	Orthogenic numerical characters = *Rectigradations*
Allometrons give rise to analogies, never to homologies; they are quantitative and intensive and not numerical; closely related forms give rise to different allometrons even within species; they may be induced experimentally in ontogeny; they constantly afford indices and ratios; even specific affinity may not predispose to the same allometrons; allometrons—both harmonic and disharmonic—frequently accompany changes of environment; they give rise both to convergence and to divergence.	Rectigradations give rise to homologies, strictly speaking homomorphic structures; they are neomorphs, new outgrowths, numerically new characters; similar rectigradations may arise in all the descendants of similar ancestors at different periods of time; they always give rise to parallelism or convergence between the members of related phyla; they are comparatively infrequent phenomena; they are not known to be produced experimentally in ontogeny; they arise from minute beginnings at different points in the tissues; they adopt the characters of proportion in surrounding parts; no true rectigradations have been observed to arise *per saltum*; the closer the taxonomic affinity the more numerous the similar rectigradations.

4. *Differences of Opinion as to the Origin of New Numerical and Proportional Characters*

In my opinion, which is not shared by all my co-workers, rectigradations and allometrons are qualitatively different characters and are attributable to different combinations of causes. For example, the additional cuspules on the teeth of *Canis*, *Alopex* and *Vulpes* are typical rectigradations; they are "characters" qualitatively different from the dolichocephaly of *Vulpes* or the relative brachy-

cephaly of *Canis*. This opinion was formed in 1905 and has in my mind been established by further research.

It is, moreover, my theoretical view that rectigradations arise from some kind of germinal predisposition or prepotency or potential homology. While the "homology" or "homomorphy" uniting these new characters seem to be due to some internal hereditary kinship between the descendants of similar ancestors, their appearance is not

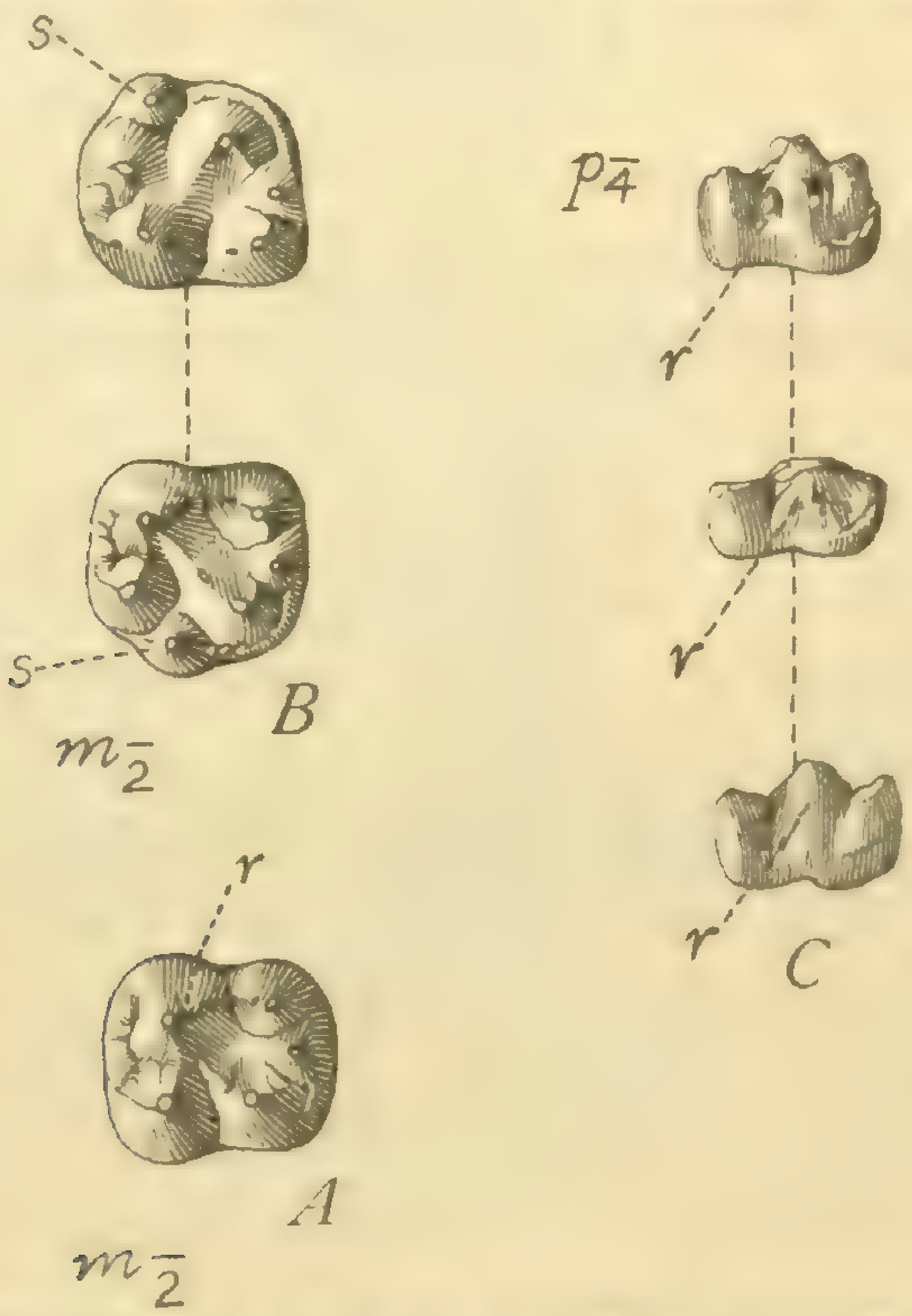

FIG. 6. Distinction between sport or mutational characters (*s*), which have no significance in the evolution of the teeth, and rectigradations (*r*), which are very important.

spontaneous, but is invoked in some way connected with similar bodily and environmental reactions which also we do not at all comprehend. For certainly there is no evidence that such "homologues" or "homomorphs" arise from similar internal perfecting tendencies or teleologic causes which operate independently of the reactions of environment and habit.

The fact that certain rectigradations appear to correspond with antecedent mechanical reactions in certain cases, such as in the cuspules of the teeth, has led to the opinion of Cope that these bodily mechanical reactions are causative, but this opinion is completely offset by the fact

that many rectigradations occur in both vertebrates and invertebrates which are not preceded by mechanical reactions in the bodily tissues, the ornamental characteristics of the shells of molluscs, for example.

In brief, the mechanical reaction hypothesis of Lamarck and Cope fails both as to the origin of certain new rectigradations and of certain new allometrons. For example, the extremely elongated limbs of certain young quadrupeds, such as young horses and young guanocos, are proportional characters which are certainly not due to the inheritance of mechanical reactions in the adults because they are entirely different from the adult proportions.

For these various reasons I have reached the opinion that, whatever the respective causes of rectigradations and allometrons may be, they are different; that is, the occasional origin of new numerical characters and the constant changes of proportion which are going on in all organisms are due to a different series of direct causes.

This divergence into matters of opinion is, however, parenthetical. Let us now return to the observation of facts which throw light upon the properties and qualities of these least characters.

5. *Observed Differences in the Origin and Inheritance of Proportional and Numerical Characters*

Origin. The fundamental distinction between the origin of rectigradations and of allometrons is well illustrated in the six phyla, I–V, of Eocene titanotheres (Fig. 5).

It is seen that similar horn rudiments and similar cusp rudiments arise independently at different geologic times in every phylum, giving rise to a great number of new homomorphic characters. On the other hand, each phylum has its peculiar and distinctive allometrons both in the bones of the skull and of the feet. These changes of proportion are so universal and so profound that by a vast system of comparative measurements it has been ascertained that every bone of the skull, of the limbs and of the feet has its differential rate of increase and decrease. Since these characters of form and proportion are real

characters and since they affect every bone in the skeleton we discover that characters of taxonomic import may be found in every one of the small bones of the wrist and ankle joints, which while less readily measurable are of exactly the same kind of value in classification as the more conspicuous changes of proportion in the skull and in the

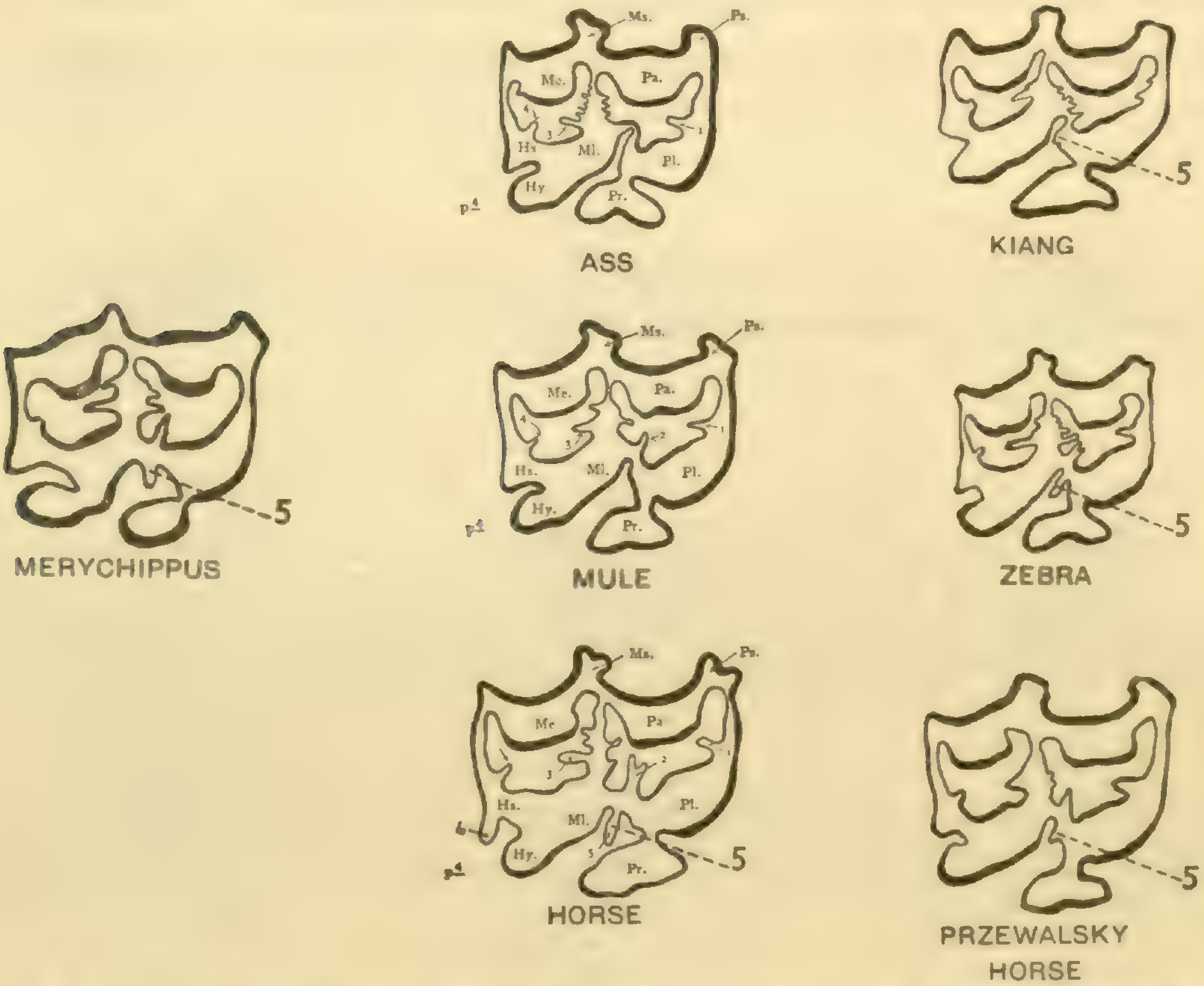

FIG. 7. The *pli caballin* (5) a typical rectigradation in the grinding teeth of different members of the family of horses. Present in the Miocene *Merychippus*, in the existing kiang, zebra, przewalsky horse, common horse; absent in the ass and the mule.

feet which Miller has used throughout in his definitions of the Carnivora. In other words a "species" may as consistently be defined by the proportion-characters of one of its carpal bones as of one of its cranial bones; such a definition would be strange and inconvenient, but it would be quite as scientific.

The rectigradations are also used in systematic definition only as soon as they become sufficiently large and conspicuous to be computed numerically. Looking up the ancient definitions of the Eocene horses by Marsh and

Cope we note in every instance that as soon as a cusp passes beyond the rudimentary stage it is apt to be observed and used in definition.

So far as we know both rectigradations and allometrons arise continuously, definitely or determinately, and so far as we have observed they arise adaptively or in an adaptive direction from the very beginning.

Inheritance. The germinal separability of the "least-characters" known as rectigradations is well illustrated in the case of the "*pli caballin,*" a delicate fold of enamel

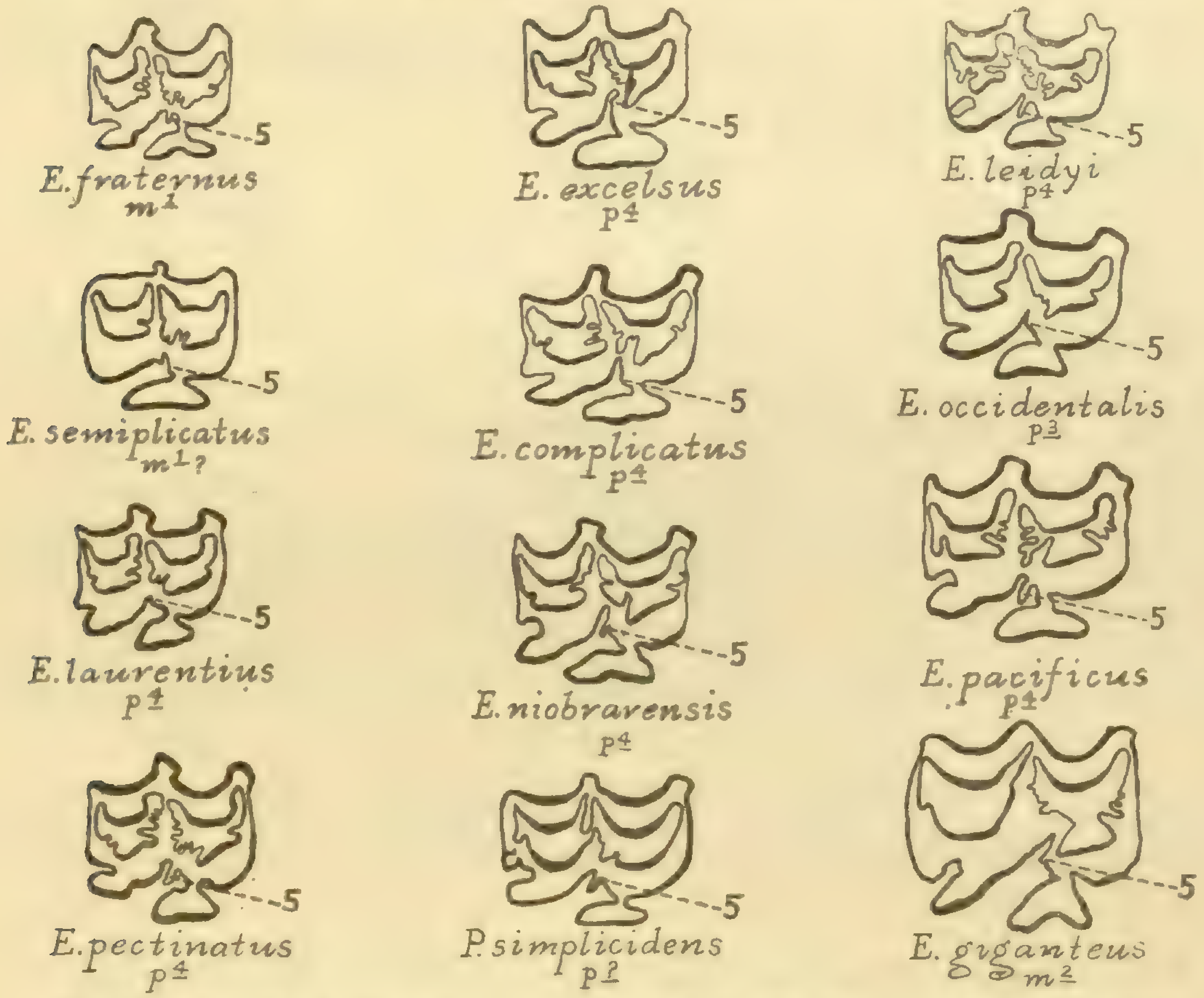

FIG. 8. The *pli caballin* (5) more or less distinctly developed in the superior grinding teeth of twelve species of the Pleistocene horses of North America. It is observed that these grinding teeth differ profoundly in the proportions of all their parts. The *pli caballin* (5) is worn off in the aged grinding tooth.

which the French systematic writers a century ago selected as a specific "character" by which the horse (*E. caballus*) could invariably be distinguished from the ass (*E. asinus*). They little knew how very ancient and stable this minute character is. We see it strongly developed in the Miocene *Merychippus.* We do not know whether it

developed gradually or suddenly within the highly varied horses of this genus. It appears (Fig. 5) more or less fully developed in all of the many known species of Pleistocene horses of America as described by Leidy, Gidley and Hay. It lies near the surface of the crown, and in much-worn teeth it disappears because the fold is seldom continued down into the lower half of the crown. It is entirely absent in the grinding teeth of the domesticated ass (*E. asinus*), yet it is present in the kiang (*E. kiang*).

The complete germinal separability of the "*pli caballin*" as a hereditary character is demonstrated by its absence in the grinders of the mule, the cross between *E. caballus* ♀ and *E. asinus* ♂; these grinders of the mule hybrid also prove that rectigradations are distinct from allometrons, because the rectigradations of the maternal horse molar are not inherited in the hybrid while the allometrons are inherited, namely, the elongated proportions of the maternal horse molar. I am preparing to investigate the grinding teeth of the hinny, the cross between the male horse and the female ass, to ascertain whether the same contrast in heredity prevails here. I suspect not because the hinny appears to have the shorter head of the ass rather than the very long horse-like head of the mule.

The germinal separability of allometrons or proportional characters of mammals is also observed, but it appears to be less complete than that of rectigradations. This is demonstrated not only in the grinding teeth but in the skull of the mule hybrid, in which the *majority* of the head proportions present the same indices as in the horse, while the *minority* of the head proportions present a blend between the indices of the horse and ass. Again in *Homo sapiens* the allometrons are in the first generation completely separated; in intermarriage of dolichocephalic and brachycephalic individuals the children do not form a blend of their parents but inherit either the pure dolichocephalic or pure brachycephalic head form. Prolonged interbreeding and intermixture between long-headed and broad-headed human races ap-

pears to break down these separable allometrons and ultimately results in blending. This may be partly due to the fact that changes of cranial proportion occur not only within the species, but within the races and sub-races of *Homo sapiens,* as witnessed in the mongoloid Indian races of North and South America. In other words, the allometrons in man are of more recent origin than in the horse and ass, which probably separated from each other as far back as the Lower Miocene. Further experiments and observations are greatly needed as to the separableness or blending of allometrons in hybrids.

As to the rapidity of evolution of proportional and numerical characters it appears that in certain lines allometrons may evolve more rapidly than rectigradations. This is seen in the titanotheres (Figs. 4, 5, 10), in which changes of proportion develop very rapidly, while the rectigradations on the grinding teeth and the rudiments of horns develop very slowly. On the other hand, in the contemporary Eocene horses the rectigradations seen in the addition of cusps develop very much more rapidly than the changes of proportion in the skull. This contrast between horses and titanotheres, however, confirms the universal law that every "character" has its differential phyletic movement as well as its differential ontogenetic movement.

That these movements are not identical is further shown by a familiar illustration. The median toes of the feet of the desert-living *Hipparion* have a much more rapid phyletic movement than the median toes in the forest-living *Hypohippus,* yet we may be sure that the limbs of the newly born foals of *Hipparion* and of *Hypohippus* were alike relatively elongated to enable these foals to accompany the mares in flight, this adaptation being secured through ontogenetic movement, or acceleration.

These differentials in the velocity of characters in their phyletic and in ontogenetic movements may afford one of several reasons why allometrons, or proportional characters are separable in hybrids, why some "unit

characters" are dominant and others recessive. This raises the general problem of the *various causes of separability of characters in the body and in the germ.* First, it will appear that continuity or discontinuity of origin has little to do with separability in the germ.

6. *Waagen's Observations on the Continuous and Orthogenic Origin of New Characters*

The first paleontologist to point out the separate origin and phyletic movement of single new numerical characters as distinguished from contemporary proportional changes was Waagen in his observations on *Ammonites subradiatus,* published in 1869 (p. 23).[14] His two great principles were announced as follows:

I. [The Variety.] The characters observed *in space* by botanists and zoologists to distinguish "local varieties," "geographic varieties," "varieties in space" are of variable value and of small systematic importance. They appear to be temporary. They do not reappear in the next higher geologic stratum. For these characters the long-used name "variety" will suffice.

II. [The Mutation.] In contrast to the *variety* I venture to propose a new term, "*mutation,*" for the early and later phases (*formen*) of a species observed *in time.* These mutations are characters which are highly constant, although minute they surely are recognizable again, on which account far greater weight must be put on mutations. They ought to be very precisely pointed out, for mutation characters even when displayed in the most minute features are certain to reappear in the next geologic stratum. In each higher stratum they show a somewhat different appearance. Ordinarily the gradations between the mutations are the more minute as the stratum from which specimens come are the more closely connected.

An ascending series of mutations in successive geologic horizons taken together constitute Waagen's *Collectivart,* which is equivalent to the *Formenreihe* of Beyrich; it is also equivalent to the *phylum* of more modern terminology. Each mutation stage includes a number of geographic "varieties." In any given geologic stratum a

[14] Waagen, W., "Die Formenreihe des Ammonites subradiatus. Versuch einer Paläontologischen Monographie," *Geognostisch-Paläontologische Beiträge,* Band II, Heft II, Nov. 1869, pp. 179–256 (Heft pp. 1–78), Pll. XVI–XX.

"mutation of Waagen" would appear as a Linnæan "species" when compared by an observer with contemporary mutations in other phyla; that is, each phylum may be separated from contemporaneous phyla by valid Linnæan characters.

The essence of Waagen's discovery is that when we observe the origin and evolution of single characters in time we are able to detect the *incipience of new characters and the profound hereditary phyletic movements*

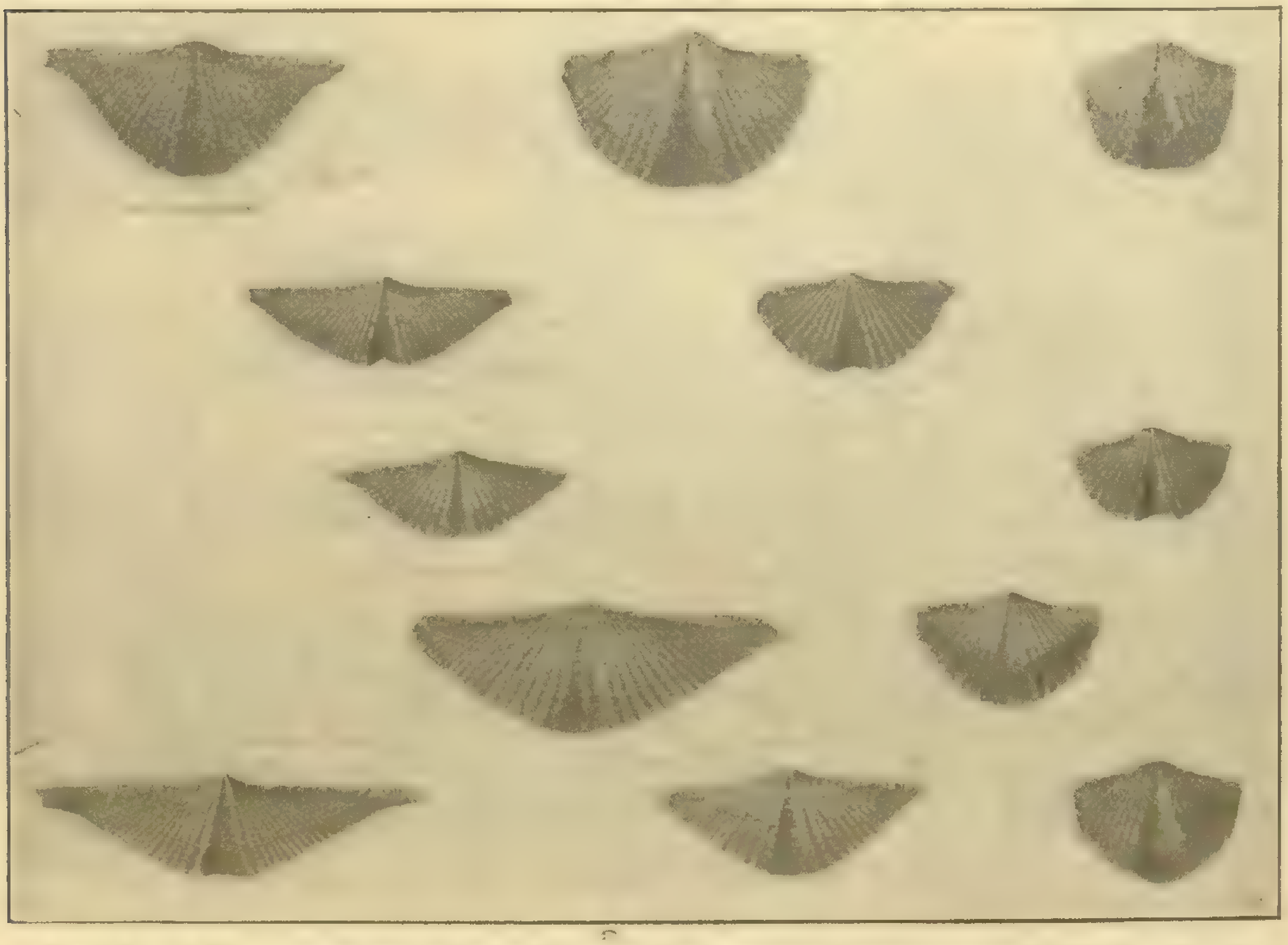

FIG. 9. "Mutations of Waagen" seen in *Spirifer mucronatus*. On horizonta lines are the geographic "varieties" differing in proportions. In vertical lines are the successive "mutations" differing in proportional and numerical characters. After Grabau.

which can not be observed by the zoologist at all. We are able, moreover, to distinguish the minute and even inconspicuous characters, which are evolving in definite directions and accumulating in successive generations, from the indefinite and transitory characters of the geographic "variety." The underlying cause of this distinction in the light of our present knowledge is that mutations denote germinal or phyletic evolution while varieties may simply denote the bodily fluctuations caused by

habit and environment. This phyletic movement was termed *Mutationsrichtung* by Neumayr.

The significance of the term mutation as defined by Waagen is to be found only in his original definition; it has been used in many different senses before and since.[15] It is a *taxonomic term* for each of the minute subdivisions of a specific phylum which may be defined by certain degrees of advance in "mutation-characters" evolving continuously in definite directions. The vertebrate paleontologist Depéret in 1907 pointed out that the Waagen "mutation-characters" have this special characteristic, they are always produced in the same direction without oscillations or retarded steps. The lacunæ are so infrequent as not to interrupt the general view of the continuity. Each of the closely linked terms of any series may be designated as "ascending mutations" in rising strata. It becomes possible to recognize the intensity of the action of time.

The mutations of Waagen demonstrate five very important principles in the evolution of certain "least characters" as follows: (1) origin from inconspicuous beginnings; (2) continuity rather than discontinuity; (3) definite direction or *Mutationsrichtung* rather than indefinite or variable evolution; (4) a definite rate of phyletic movement.

These principles appear to involve a hereditary or germinal basis, a *Mutationsrichtung,* such as also appears to underlie Osborn's rectigradations.

The two kinds of characters observed by Waagen[16] are also exactly similar to those observed by field zoologists and by vertebrate paleontologists, namely:

> The mutation of Waagen is a *stage of advance* in the development either of numerical or of proportional characters, definable when one or more of these minute and originally unimportant characters become visible and measurable.

[15] Scott, W. B., "On Variations and Mutations," *The Amer. Journ. of Science*, 3d Ser., Vol. XLVIII (Whole No. CXLVIII), Nos. 283–288, July–Dec., 1894, pp. 355–374. In this valuable paper continuous "mutation" is distinguished from indefinite "variation."

[16] Waagen, *op. cit.*

It has now been made clear why these stages are recognizable and definable by the paleontologist and not by the botanist, zoologist or experimentalist.

To sum up the observations of zoologists and paleontologists with regard to the single property of the *movement of characters* in progressive organisms the following principles are observed:

All characters are in simultaneous movement; such movements are differential, each character has its own rate; some movements are ontogenic or with a velocity-relation to other characters in the same individual; others are phyletic or with a velocity-relation to similar characters in other species and genera; "least-character" movements are continuously progressive or retrogressive; certain least-characters are stationary; "least-character" movements may develop or manifest a certain direction or trend, the *Mutationsrichtung*, and thus may be cumulative to an extreme; all character movements which are cumulative in successive generations have a germinal or hereditary basis. Thus differential movement is one of the most distinctive and important properties of the "character."

This principle of continuous germinal change which appears to underlie the continuous development of visible bodily characters may prove to be in harmony with rather than in opposition to the law through which some characters appear suddenly, or by saltation. As I pointed out many years ago, there may be an apparent but not a real contrast between the "mutations of Waagen" and the "mutations of De Vries." If the former rise through continuous germinal changes and the latter rise through discontinuous germinal changes the common element in both may be the *Mutationsrichtung*, or trend of development. In Waagen's law this trend of development appears to express itself in a continuous change of visible somatic development of characters. In De Vries's observations the change is believed to be discontinuous, suddenly constituting the "mutant"

or "elementary species" which is a subspecific stage comparable to the "mutation" of Waagen.

7. *De Vries's Observations on the Discontinuous and Indefinite Origin of Characters*

The sudden origin of "characters," new, germinal, saltatory, in every direction but of sufficient value to come under the influence of selection—these are the essential features of the famous mutation hypothesis of De Vries. While I do not accept this hypothesis as a demonstrated *natural* principle like that of Waagen, the opinions of its distinguished author may be clearly set forth as compared with the observations of Waagen which have been repeatedly confirmed and verified:

(1) De Vries's "mutants" differ from the "mutations" of Waagen in appearing as fully formed "characters" and attracting our immediate attention and observation, instead of passing through a long series of initial and rudimentary stages in which they are barely discernible. (2) The "mutation-characters" observed by De Vries differ from Waagen's mutation-characters in lacking any definite or determinate direction; on the contrary, it is of their essence that they appear in any or all directions. (3) They agree, however, with the "mutation-characters" of Waagen and with the rectigradation-characters of Osborn in the fact that similar mutations may arise independently at various times in branches of the same stock, thus giving rise to homomorphic characters. (4) Let us note that the new systematic unit of De Vries, the "mutant" or "elementary species," is a *space or geographic phenomenon;* it may be contemporary with many other mutants of a single Linnæan species. (5) The "mutation-character" of De Vries is not a demonstrated equivalent to the "mutation-character" of Waagen, which is a *time or geologic phenomenon* character, observable only in a long series of generations from the same ancestor.

Now as to the present state of evidence for the saltation

hypothesis of De Vries, let us be on our guard between *fact* and *opinion,* between *natural* and *unnatural* phenomena. That the saltation of characters occurs very frequently in plants and in animals under artificial conditions there can be no doubt; yet the mass of existing evidence is from *artificial* rather than from *natural* sources.

The recent opinion of Bateson,[17] who by his advocacy of discontinuity in the origin of all specific characters would be predisposed to favor the saltation theory held by De Vries, is partly negative.

> The evidence for the appearance of mutations of higher order, by which new species characterized by several distinct features are created, is far less strong, and after the best study of records which I have been able to make I find myself unconvinced. . . . In so far as mutations may consist in meristic [i. e. numerical][18] changes of many kinds and in the loss of [germinal][18] factors it is unnecessary to repeat that we have obtained evidence of their frequent occurrence.

Negative conclusions have also been reached by various botanists, as, for example, Jeffrey:[19]

> 1. The Onagraceæ are largely characterized by hybrid contamination in nature.
>
> 2. This statement holds with particular force for *Œnothera lamarckiana* and other species of the genus *Œnothera,* which have served as the most important basis of the mutation hypothesis of De Vries.
>
> . . . .
>
> 7. The mutation hypothesis of De Vries, so far as it is supported by the case of *Œnothera lamarckiana,* is invalidated.

I do not know of a single instance where a field observer in mammalogy or in paleontology has recorded a new saltation character which is known to be of any significance in the evolution of the race. On the other hand, certain field observers of birds (Beebe) and of molluscs (Crampton) are of the opinion that they have discovered proofs that certain characters arise by saltation

[17] Bateson, William, "Problems of Genetics." Oxford University Press, 1913, 250 pp.

[18] These [] are insertions in Bateson's text by the present author.

[19] Jeffrey, Edw. C., "Some Fundamental Morphological Objections to the Mutation Theory of De Vries," THE AMER. NATURALIST, Vol. XLIX, No. 577, Jan., 1915, pp. 5–21.

in a state of nature. The vast majority of observations on the evolution of mammals either in the field (*e. g.*, Osgood) or among fossil series, where the *intergradations* have not been destroyed, points to continuity in the origin of changes of proportion. The evidence as to this continuity both in proportional and in certain numerical characters in fossil vertebrates and invertebrates is overwhelming. Saltation is, however, theoretically probable in certain numerical and meristic characters, such as supernumerary teeth and vertebræ.

8. *The Separability of Characters in the Body*

Apart from the question of the origin of new characters by saltation, the observations of De Vries and his followers furnish additional evidence of the *separability of characters* both in the body and in the germ. This law of separability in its human aspect was well expressed by Galton in 1889.[20]

> . . . We seem to inherit bit by bit, this element from one progenitor, that from another, under conditions that will be more clearly expressed as we proceed, while the several bits are themselves liable to some small change during the process of transmission. Inheritance may therefore be described as largely if not wholly "particulate," and as such it will be treated in these pages. Though this word is good English and accurately expresses its own meaning, the application now made of it will be better understood through an illustration. Thus, many of the modern buildings in Italy are historically known to have been built out of the pillaged structures of older days. Here we may observe a column or a lintel serving the same purpose for a second time, and perhaps bearing an inscription that testifies to its origin, while as to the other stones, though the mason may have chipped them here and there, and altered their shapes a little, few, if any, came direct from the quarry. This simile gives a rude though true idea of the exact meaning of Particulate Inheritance, namely, that each piece of the new structure is derived from a corresponding piece of some older one, as a lintel was derived from a lintel, a column from a column, a piece of wall from a piece of wall.
>
> I will pursue this rough simile just one step further, which is as much as it will bear. Suppose we were building a house with second-hand materials carted from a dealer's yard, we should often find considerable portions of the same old houses to be still grouped together. Materials derived from various structures might have been moved and much shuffled together in the

[20] Galton, Francis, "Natural Inheritance." 8vo. The Macmillan Company, 1889.

yard, yet pieces from the same source would frequently remain in juxtaposition and it may be entangled. They would lie side by side ready to be carted away at the same time and to be re-erected together anew. So in the process of transmission by inheritance, elements derived from the same ancestor are apt to appear in large groups, just as if they had clung together in the pre-embryonic stage, as perhaps they did. They form what is well expressed by the word ''traits,'' traits of feature and character—that is to say, continuous features and not isolated points.

The observations which we have been comparing on the origin of new characters in vertebrates, invertebrates and plants certainly afford some insight into the laws of germinal change wherever we can distinguish between the true germinal expression of a character and its visible modification by ontogeny or environment. Recurring to the antithesis between the separability and the correlation of characters we may again sum up some of the many properties and qualities of single characters:

Laws of Separability

Independent germinal origin of characters, either continuous or discontinuous; independent development of similar new characters in near or remotely related descendants of the same ancestors; development of dissimilar changes of proportion in descendants of nearly related ancestors; development of similar "rectigradations," "mutations of Waagen," and "mutations of De Vries" at different times in descendants of the same ancestors; separate rate of evolution in ontogenetic movement and in phyletic movement; each character with its own origin, individuality, and rate of movement.

Laws of Correlation

Hereditary connection with the origin of similar characters in other lines of descent; strong sex correlation in the size and proportions of all characters; mechanical correlation both in the allometrons and rectigradations; compensation correlations when one character is developed by the sacrifice of another; proportional or allometric correlations where all the new characters and changes of proportion share the general form; a utility or selection correlation where one character is sufficiently strong to come directly under natural or sexual selection; adaptive, defensive and offensive correlation where groups of characters evolve to serve similar purposes.

At the present time we appear to understand the laws of separability somewhat better than the laws of correlation, for the latter are enveloped in the deepest mystery. We have not even alluded to the chemico-physical

correlations, by means of the hormones, which are observable in the field of physiology and medicine. Mechanical correlation, as where an incipient rectigradation in an upper tooth corresponds precisely with an incipient rectigradation in a lower tooth so that the upper and lower teeth evolve throughout in perfect mechanical harmony by the addition of character after character, affords one of the most vivid instances of our total ignorance of the causes underlying the origin of new characters.

9. *The Separability of "Characters" in the Germ*

The pioneer discoverer of the separability of "characters" in the germ was Gregor Mendel, who in 1865 examined seven pairs of alternating characters in hybrids of two varieties of the common pea (*Pisum sativum*). The outstanding feature of this great discovery is that the separability of the "determiners" of characters in the germ is far more sharply defined than the separability of the corresponding somatic "characters" in the visible body; as regards the "determiners" of many characters it is sharp and absolute.

A second feature of Mendel's discovery is of special significance in our present review of proportional and numerical characters in the hard parts of vertebrates because the alternative characters which Mendel experimented with were of both the proportional and the numerical kind. For example, Mendel's proportional characters of "tallness" and "dwarfness" in the pea stalk may not improperly be regarded as analogous with the alternative characters of dolichocephaly and brachycephaly of the skull of the mammal. Again, Mendel's form of the pea-seed, whether round or wrinkled, may not improperly be compared respectively with the folded or smooth condition of the enamel in the molar teeth of the ass and of the horse, which we observe is alternative in heredity. On the other hand, Mendel's definite color characters, such as his flower color, whether purple, red or white, or his seed color, whether yellow or green, have been definitely compared by experiment and found to

correspond in heredity with the coat colors of mammals.

A third great feature of Mendel's discovery is that in such alternative pairs of characters as tallness and dwarfness one may be dominant and the other recessive, following in successive generations the well-established Mendelian law.

It remains to be observed whether any of the *proportional* characters of mammals follow this law.

It remains to be observed, also, whether rectigradational characters like the "*pli caballin*" in the grinding teeth of the horse exhibit in heredity the Mendelian laws of dominance and recession. This could only be ascertained among mammals by observations on hybrids in such a family as the Bovidæ, which are fertile *inter se*,[21] or on similar characters in the hybrids between wild natural breeds.

The discovery through experiment by zoologists and botanists that many saltation characters, such as sports and abnormalities, follow the Mendelian law of dominance and recession has led to the entirely unwarrantable assumption that only characters which are discontinuous, or of sudden origin, are sharply separable in heredity. This we have seen to be not in accord with the facts, for the *principle of separability is quite as sharply defined in certain characters which have evolved slowly and continuously as in those which have evolved suddenly.*

The special significance of recent Mendelian discov-

[21] Bartlett, A. D., "Wild Animals in Captivity." 8vo. Chapman and Hall, Ld. London, 1899, p. 219. "The two species of camel (*Camelus dromedarius* and *C. bactrianus*) will breed together; the llama (*Auchenia glama*) will breed with the alpaca (*A. pacos*), and the offspring are fertile. Several species of deer, when crossed, produce fertile hybrids: for instance, the Barbary deer (*Cervus barbarus*) with the red deer (*C. elaphus*), the Mexican (*C. mexicanus*) with the Virginian deer (*C. virginianus*). Several others are also recorded upon good authority. Several instances of hybrids among the carnivora are well authenticated. The lion (*Felis leo*) has bred with the tiger (*F. tigris*), the leopard (*F. leopardus*) with the jaguar (*F. onca*), the wild cat (*F. catus*) with the domestic cat (*F. domestica*)."

"Wild Beasts in the 'Zoo.'" 8vo. Chapman and Hall, Ld. London, 1900, pp. 71–72. "Hybrid Bovine Animals. . . . In the first place, the bull

eries to the zoologist and paleontologist is that what we zoologists describe as a "single character," the horn of a sheep or of a titanothere, for example, is probably the expression of a very large number of germinal "determiners" all of which are necessary in the germ to produce the horn. If only one of these "determiners" should change the character of the horn would change, or the horns might be lost altogether. Thus the germinal correlation of "determiners" to produce what appears as a single character to the zoologist and paleontologist is visibly represented in the bodily correlation of the horn with a very elaborate offensive and defensive mechanism including all the bony and muscular characters necessary to operate the horn effectively, as well as the psychic desires and impulses to use the horn. We thus have a vast array of internal and external, of structural and functional correlations with this single character, the horn.

10. *Conception of the "Least Character"*

In the beginning of this address I noted that no one has ever undertaken to define the "character."

It is very difficult if not impossible to define one of these least characters as observed in living and fossil series even when reinforced by the experimental evidence of heredity. A definition may be essayed through

Zebu (*Bos indicus*) was introduced to the cow Gayal (*Bibos frontalis*). and a female hybrid was born October 29, 1868 (*A* of pedigree). This animal (*A*) produced her first calf June 17, 1872, a second one October 16, 1873, a third one January 5, 1875, a fourth March 11, 1876, a fifth November 2, 1878; these five calves were the produce of this female hybrid Gayal with the Zebu bull. She was now introduced to the male American Bison (*Bison americanus*), and on May 21, 1881, she produced a female No. 2 (*B* of pedigree)."

"It will be seen that this animal (*B*) is the product not only of the intermixture of three well-marked species, but, according to our present definition, of three distinct genera.

"This remarkable animal, the result of the triple alliance, was last year introduced to the bull Bison, and on March 12, 1884, she produced a female (*C* of pedigree). This last individual, now eleven weeks old, is undistinguishable from a pure-bred Bison of the same age."

an enumeration of the known properties of a "least character."

As distinguished from a group of characters the properties of a "character" are its separability, its independence, its individuality, its own rate of movement ontogenetic and phyletic, its differentiation by these properties from other least characters. Its separability in heredity is shown where it can be hybridized.

From the structural or anatomic standpoint a least character is a group of cells and tissues constituting a diminutive organ or part of an organ subserving a distinct though subsidiary function. For example, the "*pli caballin*" in the enamel of the horse's tooth or the rudimentary cuspule may be cited as least characters, for each is composed of a vast number of cells and more than one tissue, but seems to have the property of rising or falling and behaving like a unit.

11. *"Least Characters" in Classification and Systematic Work*

This "least-character" conception is of great value to the zoologist and botanist in systematic work, this conception of an individual as a colony of "characters" each with its principles of independence and its principles of correlation, germinal in origin but subject to somatic modification by environment and habit.

First, among these single characters are those observed by Waagen which accumulate until they build up into one of his "mutations." One or more such single characters compose the "mutants" of De Vries.

Second, the old but oft-confusing term "races" becomes clear; we may now understand the significance of the races of the horse, for example, the Arab, the Forest, the Steppe horse. These races are all fertile *inter se* and thus have never been defined as species although fertility and non-fertility are no more important in the distinction of species than any other "character." The characters which distinguish races are, nevertheless, often of specific value; they are either proportional or numer-

ical, because in the production of these modern races the pure ancestral forms had in their natural state evolved a very large number of allometrons as well as rectigradations and other numerical characters which have to a slight degree blended in intercourse and to a larger degree have maintained their purity and distinctness. Thus you will observe among the modern races of horses the most incongruous mixtures; an old cart horse with the head and quarters of the Forest type will gallop across a field and raise the bones of the tail perfectly erect exactly like a pure bred Arab.

Similarly a "species" is a mosaic of an infinite number of least characters in a state of movement only a few of which may be so definite and measurable as to be employed in systematic definition.

12. *Theoretical Conclusions as to "Characters" and the "Organism"*

These least characters when assembled in an organism and dominated by the principles of separability and correlation present to our fancy the picture of a vast regiment of soldiers walking in single line; each soldier possesses his own individuality and separableness from his comrades, each advances or lags behind according to his individual velocity, but each subserves the general purposes of the entire regimental line through the uniting force of training and the unseen spirit of the regiment, which represents the law of correlation.

It appears that we paleontologists have already learned much and that we have still far more to learn by the closest observation of "characters" in a state of *natural evolution*. We are on somewhat safer ground than the observer of the unnatural or hotbed evolution of characters in the artificial breeds, hybrids of animals and plants under domestication. The contrast between the excessively slow natural evolution during the past million years of the wolf, the arctic fox and the red fox, and the feverish unnatural evolution of the domestic breeds of dogs dur-

ing the last ten or twelve thousand years is extremely significant.

If the student of genetics abandons the natural and the

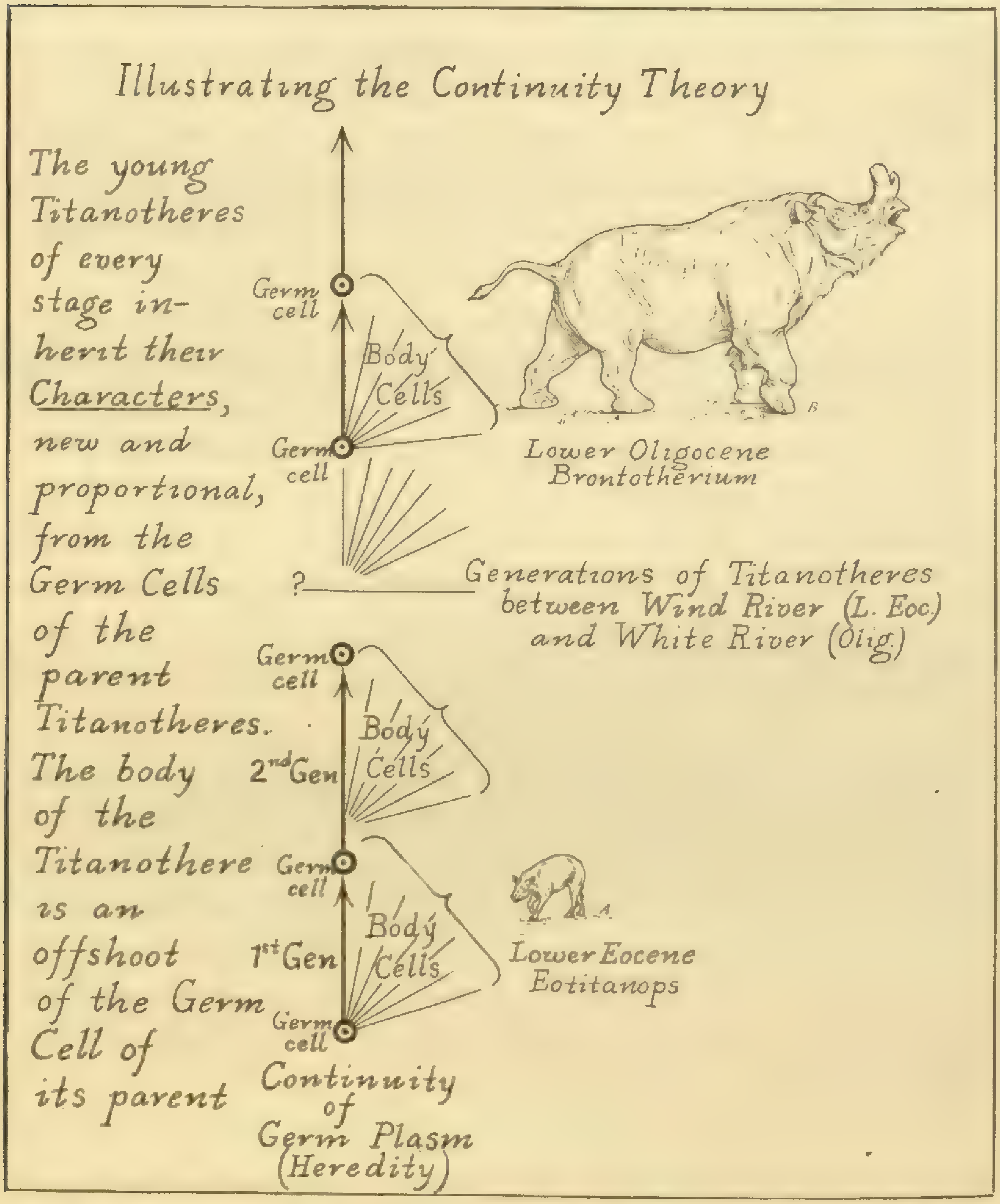

FIG. 10. Evolution by the constant addition of characters, illustrated in the descent of *Brontotherium* (*B*) from *Eotitanops* (*A*). These animals are represented to the same scale. They illustrate the constant addition of new characters in every part of the organism. Evolution in this family of quadrupeds is almost entirely by the addition of characters. Comparatively few characters degenerate or disappear.

normal for the unnatural and the abnormal and sticks solely to his seed pan and his incubator he is in danger of observing modes of origin and behavior of characters

which never have and never will occur in Nature. He may, moreover, never observe at all certain modes of origin and behavior as well as certain properties and qualities of characters which are of the most fundamental importance in relation to his particular field of heredity and hybridizing.

While twenty years of observation of the normal and the natural aspects of nature have brought the zoologist and paleontologist somewhat nearer to a conception of the modes of evolution, twenty years of continuous observation of the abnormal and unnatural have landed one of the leading experimentalists, William Bateson, in the state of skepticism and agnosticism expressed in his recent work (p. 248, italics our own):[22]

> The many converging lines of evidence point so clearly to the central fact of the origin of the forms of life by an evolutionary process that we are compelled to accept this deduction, *but as to almost all the essential features, whether of cause or mode, by which specific diversity has become what we perceive it to be, we have to confess an ignorance nearly total.* The transformation of masses of population by imperceptible steps guided by selection, is, as most of us now see, so inapplicable to the facts, whether of variation or of specificity, that we can only marvel both at the want of penetration displayed by the advocates of such a proposition, and at the forensic skill by which it was made to appear acceptable even for a time.

If the principle of the continuous and independent movement of each member of a vast colony of single characters is firmly established, as it appears to be through vertebrate and invertebrate paleontology, we must abandon entirely one tradition left by the master mind of Darwin which has permeated the work of all the original Darwinians and Neo-Darwinians, and which is equally strong in the mind of De Vries. Bateson has recently maintained this tradition of the origin of "species" from fortuitous saltatory characters in the following language.[23]

[22] Bateson, William, "Problems of Genetics." 8vo. Oxford University Press, 1913, 250 pp.

[23] *Op. cit.*, p. 248. Italics our own.

In place of this doctrine we have little teaching of a positive kind to offer. We have direct perception that new forms of life may arise sporadically, and that they differ from their progenitors quite sufficiently to pass for species. *By the success and maintenance of such sporadically arising forms, moreover, there is no reasonable doubt that innumerable strains, whether in isolation or in community with their co-derivatives, have as a fact arisen, which now pass in the lists of systematists as species.*

Broadly stated, this tradition is that evolution manifests itself suddenly in one character or group of characters; that either through individual variation such a character or group of characters is preserved and accumulated by selection, or, through saltation that such a character or group of characters suddenly arises and is imperishably fixed in the race by selection.

This is the essential feature of the Darwinian conception of evolution, namely, that an organism advances now here, now there. Such a conception is one which would naturally be fosterd by observers of single living plants or animals living under unnatural conditions, or by experimentalists who observe a brief contemporary chain of organisms.

The observation of "characters" in phyla or groups of organisms advancing on a grand scale in space or in time shows that this Darwinian tradition is so partial and inadequate as to be practically false. It has been observed that every organism consists of an almost infinite number of characters, it has also been observed that the evolution of some of these characters may be so conspicuous as for a time to attract the attention of the observer or as to constitute the chief magnet for the power of selection. It has not been observed that the entire organism waits on any one of these characters. On the contrary, in all progressive organisms in which a very large number of characters are simultaneously observed it proves that every character in every part of the body is in a continuous state of movement. This is the actual result of observation and measurement.

As regards natural selection in relation to the *origin* of

characters we know nothing, we stand by the theoretic opinion that: *Selection is operating always upon the sum of all the movements, actions and reactions of characters known as the* ORGANISM *and upon all single characters of survival or elimination value.*

Very recent is Bateson's enunciation[24] of the novel hypothesis that we may have to forego the theory of addition of germinal factors or determiners and substitute a theory of variation by loss of "factors":

Paleontology affords only indirect evidence as to germinal "factors" but it offers the most positive testimony both as to evolution largely by the loss of characters, as in the case of the family of horses, and evolution largely by the addition of characters, as in the family of titanotheres displayed in Fig. 10. It is the constant addition of new somatic characters in the evolution of members of the latter family which forms the background of the present address. Whether the incessant and most impressive addition of the new somatic characters which transform *Eotitanops* into *Brontotherium* are the visible result of a subtraction of germinal "factors" may be a subject for metaphysical discussion, but is certainly without the bounds of all natural evidence. A natural view is that the invisible germ is being continuously enriched with the visible body by processes of which we can form no conception whatever.

[24] Bateson, Wm., "Heredity." Inaugural Address of President to The Australian Meeting of the British Association. *Nature*, Vol. 93, No. 2338, Aug. 20, 1914, pp. 635–642. (Italics our own.) "I feel no reasonable doubt that though we may have to forego a claim to variations by addition of factors, yet variation both by loss of factors and by fractionation of factors is a genuine phenomenon of contemporary nature. If, then, we have to dispense, as seems likely, with any addition from without *we must begin seriously to consider whether the course of evolution can at all reasonably be represented as an unpacking of an original complex which contained within itself the whole range of diversity which living things present.* I do not suggest that we should come to a judgment as to what is or is not probable in these respects" (p. 640).

THE INFERTILITY OF RUDIMENTARY WINGED FEMALES OF DROSOPHILA AMPELOPHILA

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WHILE the infertility of the females of the mutant stock of *Drosophila* called rudimentary, was apparent from the beginning, the cause of the infertility was uncertain. Many rudimentary females bred to males of their own kind gave no offspring. The males with rudimentary wings, on the other hand, were perfectly fertile with wild females and with females of other stocks. The results might seem to show that sperm bearing the factor for rudimentary could not fertilize the eggs carrying the same factor. But that this was not the entire explanation was evident: for, heterozygous females fertilized by rudimentary males gave rudimentary females and males as well as long winged flies. In the heterozygous females, however, the egg, up to its maturity, has developed under the influence of the normal allelomorph of rudimentary, as well as of the rudimentary factor. I suggested,[1] therefore, that, due to this difference during the ripening period, the rudimentary bearing egg of the heterozygote could be fertilized by the rudimentary sperm, although the egg of the rudimentary female itself could not succeed in this combination, but I have never felt satisfied with this tentative explanation; for, there were other possibilities, not sufficiently studied, that might affect the result. For instance, it was not actually observed that the rudimentary males copulate successfully with females of their own kind, although it was known that they could mate with any other females. This question had first to be settled by direct observation.

Rudimentary winged females were isolated for three

[1] Morgan, *Zeit. f. indukt. Abs. und Vererb.*, VII, 1912.

days after hatching, and then each was mated to a rudimentary winged male that had similarly been isolated. In about twenty minutes, on an average, mating occurred in an entirely normal manner. The females that had mated were kept each in a separate bottle and given the best food. Examination showed that hardly one of the females had laid eggs; but in the rare cases where a few eggs were observed, some flies developed. In the first experiment seventeen females were seen to mate, and were then kept alone, or with their mates. One female produced one rudimentary winged son; another gave one rudimentary winged daughter and one such son. These three flies were the total output of seventeen females.

The next point was to determine whether these seventeen females were infertile only with their own kind of males. Each was again paired, this time to a male with bar eyes. The character bar eye is dominant. If sperm of these males should be successful, the female offspring from this cross would have bar eyes, and could be distinguished from any others that might come from the first mating. One female gave one bar daughter; another female also had one bar daughter. These results show that the rudimentary females were no more successful with bar males than with their own kind.

In a second experiment eleven rudimentary-winged females were tested with rudimentary males. One gave one rudimentary daughter and one rudimentary son, but also two long-winged daughters. Since I had not taken the same care here (using twenty-four-hour flies) as before to be certain that the females were virgin, these two long-winged daughters are supposedly due to fertilization before isolation, since long-winged males were hatching at the time in the parent stock. I tested this supposition by mating the two females to a rudimentary male, and obtained the following kinds of offspring:

Long ♀	Long ♂	Rudimentary ♀	Rudimentary ♂
56	64	15	23

Evidently then the rudimentary females had been fertilized by a long-winged brother before isolation, as well as by the rudimentary male later. No offspring were produced by a third mating, to bar males.

A third and similar experiment was made. Of twelve females, none gave any rudimentary offspring, two gave three bar daughters apiece, but no rudimentary sons.

In a fourth experiment thirteen rudimentary-winged females were isolated from stock. They were not necessarily virgins. One gave a rudimentary-winged son; another gave two rudimentary daughters and six such sons.

If only a single pair of flies is present in a culture and few or no larvæ are produced, the banana generally decays instead of fermenting. It might happen under these conditions that the few larvæ from rudimentary females might fail to develop, and the rudimentary sons might also suffer, even when some of their long-winged sisters (the father being a normal male) succeed in developing. To make conditions favorable in this respect I proceeded as follows: A red-eyed rudimentary female was kept at first with a red-eyed rudimentary male for three or four days. Then a few old white bar females and males were added and fresh food given. The presence of these flies and their progeny would serve to keep the food in good condition. Moreover if the rudimentary female had been fertilized by the rudimentary male she would produce rudimentary *daughters* and *sons.* If she were subsequently fertilized by the white bar males she would also give red (heretozygous) barred daughters, but these could not be distinguished from the daughters that the white-bar female would give were she fertilized by the red rudimentary males. Nevertheless all of the sons of a rudimentary female would be rudimentary round-eyed males, regardless as to which male was their father, and their presence would show to what extent the rudimentary females were fertile. The experiment was varied and simplified by removing the rudimentary males, when the white bar males and females were added.

One hundred and two rudimentary females were tested in these ways. In only three cases were rudimentary males produced. One female produced two; one female produced four, and another female produced one male. It is evident, therefore, that the scarcity of rudimentary sons can not, in general, be ascribed entirely to the conditions of the food.

As pointed out above, red bar daughters might appear in the foregoing tests and such females might have either of the two parentages specified. Most of the females of this kind would be expected to come from white bar females by rudimentary males, since the converse case would rarely be realized. Seven females, that appeared, were tested by breeding to white bar males and gave the results in the first of the two following tables. Four others were tested by breeding to rudimentary males with the result shown in Table I*a*. The results confirm the expectation

TABLE I

F₂, Red, Long Bar (heterozygous) ♀ by White Bar ♂

Red Long Bar ♀	White Long Bar ♀	Red Rud. Round ♂	White Long Bar ♂	Red Long Bar ♂	White Rud. Round ♂	Red Rud. Bar ♂	White Long Round ♀	Red Long Round ♂	White Rud. Bar ♂
55	44	13	24	27	7				
30	30	3	14	9	2				
65	61	3	27	10	5			2	
85	71	17	34	28	19				
48	53	15	27	21	17				
37	35	5	25	13	9				
12	10	1	5	5	0		1		1
342	304	57	156	113	59		1	2	1

TABLE I*a*

F₂, Red, Long Bar (heterozygous) ♀ by Rudimenatry ♂

Red Long Bar ♀	Red Rud. Round ♀	Red Long Round ♀	Red Rud. Round ♂	White Long Bar ♂	Red Long Bar ♂	White Rud. Round ♂	Red Rud. Bar ♂	White Long Round ♂	Red Long Round ♂	White Rud. Bar ♂
22	15		14	13	14	6			1	
37	13	1	17	26	16	9				
65	10		9	26	25	24				
56	25		25	12	14	12				
180	63	1	65	77	69	51			1	

in regard to the character of these females. Three pairs of factors are involved which should give the following classes of males:

Non-cross-overs	*Single Cross-overs*	*Double Cross-overs*
Red, rud., round.	Red, long, bar.	Red, long, round.
White, long, bar.	White, rud., round.	White, rud., bar.
	Red, rud., bar.	
	White, long, round.	

It will be observed that the rudimentary males run far behind their schedules, due beyond doubt to their poor viability.

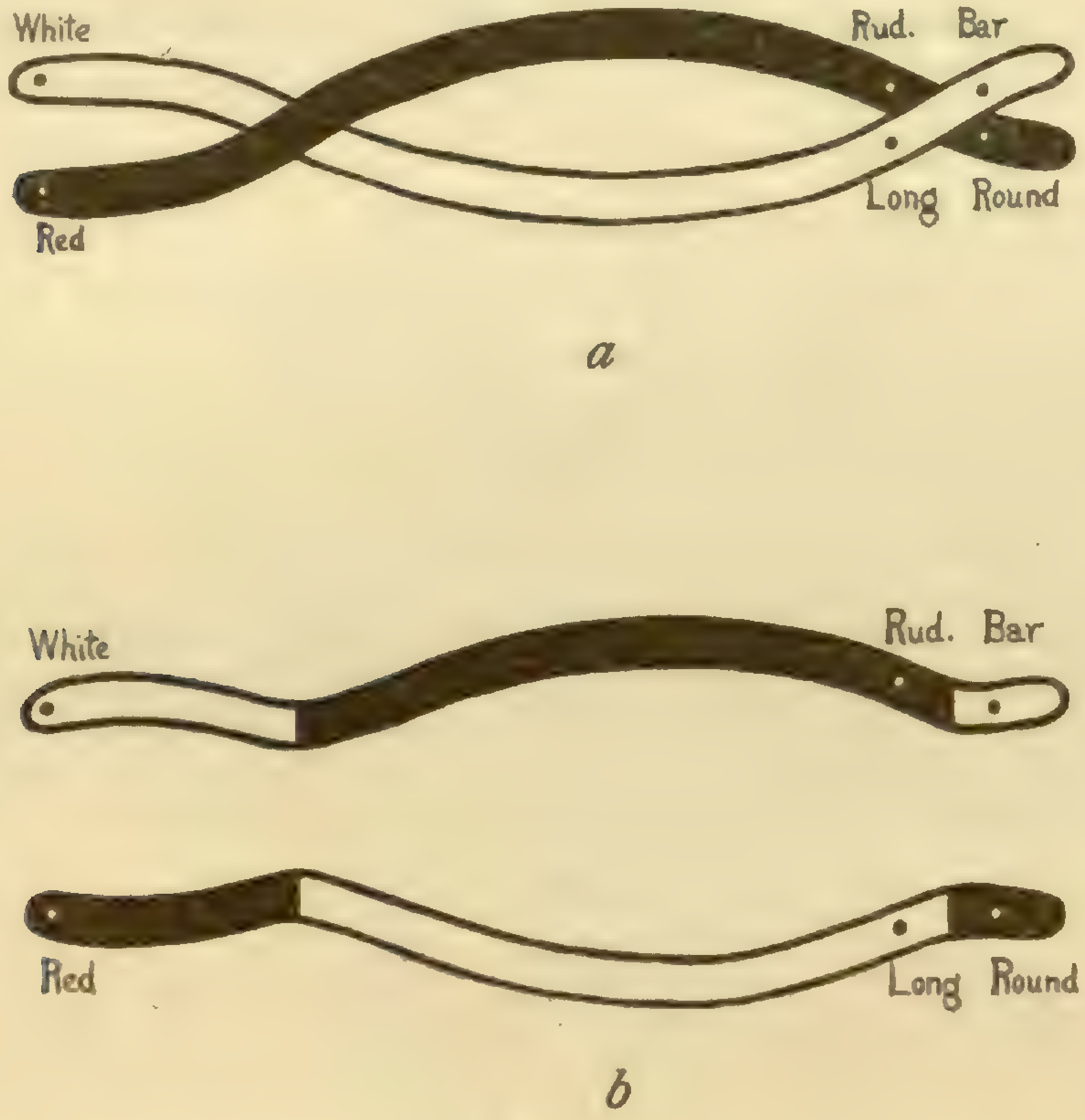

FIG. 1.

Double crossing-over took place four times in the experiment. If the two X chromosomes that carry respectively the factors for red rudimentary round and white long bar are represented as twisted once around each other, as in text-fig. 1, *a*, the result of fusion and recombination at the crossing points would give the two chromosomes shown in 1, *b*. One chromosome now carries the factors for red, long, round, and the other the factors for white, rudimentary, bar. As the tables show there are

four males that fall into these two classes. There is one female in the second table, that is red, long, round. She must have resulted from a cross-over gamete, a long, round egg being fertilized by a female producing sperm of the rudimentary male.

Still another experiment like the last one was made, but vermilion-eyed flies instead of bar-eyed flies were added. The virgin rudimentary females that were used were not allowed to mate first (as before) with rudimentary males, in order to meet a possible objection to the preceding experiment, namely that the spermathecæ, if first filled with sperm from the rudimentary males, might be incapable of filling again with sperm when another male of a different stock is added. The vermilion (long-winged males) would give, with their own females, flies with vermilion eyes, while the vermilion males that mated with the rudimentary females should give red-eyed, long-winged females and rudimentary red-eyed males. In the following table the number of rudimentary females tested in each culture is given in the top line; in the second, central, line the number of red, long offspring given by the flies in the square above; and in the lowest line a record of the number of vermilion offspring given by the flies in the culture under observation.

Number of rudimentary ♀ tested	1	6	1	1	1	7	6	1	6	5	4	6	15	6	4	4
Red long daughters		7				1	1								5 ♀, 3 ♂	
Vermilion ♂ and ♀	18	463	76	119	105	121	264	637	233	28	6	54	179	0	167	46

Out of a total of seventy-four rudimentary females only nine red-eyed daughters were produced (if we exclude one culture in which five red long females and three red-eyed males appeared which must be due to error or to contamination). Of the nine offspring seven came from one culture, and possibly from one female in that culture that laid an exceptionally high number of eggs. The complete absence of rudimentary males may be explained

as a result of competition, for, as Morgan and Tice have shown, such males tend to disappear if too many other larvæ are present.

Lastly sixty-eight more rudimentary females from stock were tested with bar males. They gave twenty-four long bar (heterozygous) females, two rudimentary round males and one mosaic that will be described below. In one bottle there had been twenty-seven rudimentary females and an examination of their food showed over forty eggs present. Since the eggs are not easily found I estimate that probably a hundred eggs were present. Out of these eggs sixteen females and one male developed (included in the total given above). It appears then that many of the eggs laid by the rudimentary females do not develop.

The condition of the ovaries of the eight surviving rudimentary females showed that seven were full sized and contained mature eggs. The mosaic that appeared in one of the last crosses (Fig. 2) is interesting in several ways. Genetically it is a female, externally it is a male in appearance, in reality it is a male in part and a female in part although the egg must have been fertilized by a female-producing sperm. On the right side of the body the eye is heterozygous for bar, there is no sex comb on the fore leg, the spines on the thorax are long, and the wing is large. On the left side the eye is pure bar, there is a sex comb on the foreleg, the spines on the thorax are short, and the wing is small. The difference in size of the two wings, and of the spines, is a characteristic difference between the male and female, connected with a difference in body size. The abdomen is pigmented above as in the male, and below there is a normal penis.

Despite the apparently normal male copulatory organs, the mosaic, when placed with mature, unmated females, paid not the slightest attention to them, although it was quite active. Of course its organs of perception were female on one side of the anterior end, although male on the other side. What physiological complex this might give, is, of course, problematical. The mosaic died by

becoming stuck to the glass before its behavior towards males could be studied.

There are two ways in which this mosaic can be ac-

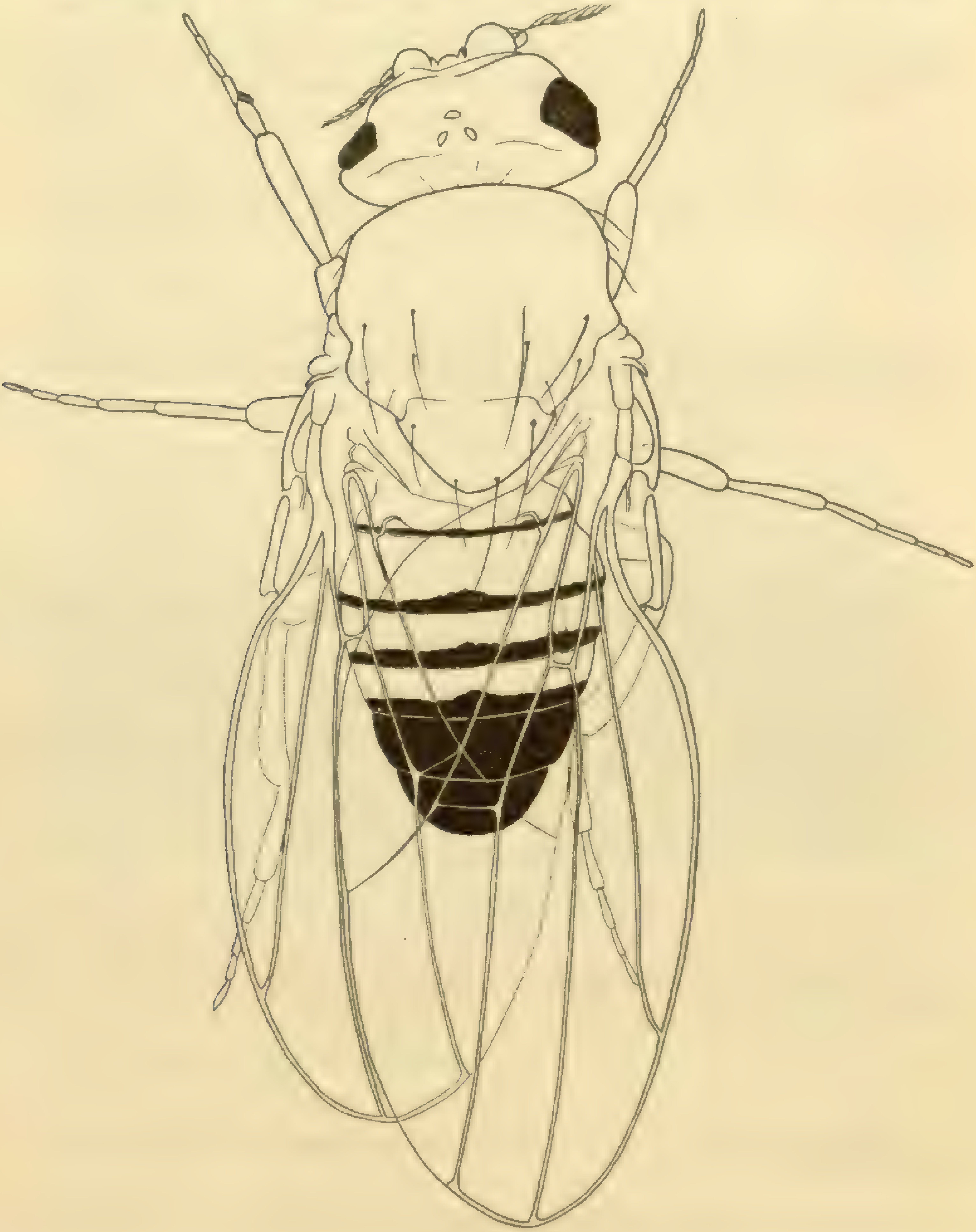

Fig. 2.

counted for. If an egg of the round-eyed, rudimentary female was fertilized by a female producing spermatozoon of the bar-eyed, long-winged male the result should

be a bar (heterozygous) eyed, long-winged female, since these are the dominants. If, then, after fertilization, dislocation of the X chromosomes occurred at some early division, so that while the X carrying the factor for bar and long divided normally (each daughter nucleus getting its proper half), the other X chromosome carrying the factors for round eye and long wings failing to divide (or else one half failed to go to one daughter nucleus) the characteristics of the mosaic can be accounted for. On the male side, the left, there would be one X chromosome in each cell, that carries the factors for bar eye and long wings. The size of the wing and of the spines, and the presence of the sex comb are a consequence of the "maleness," resulting from the presence of only one X. On the female side, the right, there would be two X chromosomes in all of the cells, hence the heterozygous nature of the bar eye. The length of the wing (the female being larger than the male) and the absence of the sex comb are a consequence of the "femaleness," due to the two X combination. The fact that the posterior end of the abdomen is purely male is owing to this region coming from the male contingent of nuclei, that must have overlapped to the right side in this region.

The other explanation of the mosaic is that two female producing nuclei entered, one alone giving rise to the male side, the other one uniting with the egg nucleus giving rise to the female side. Boveri's explanation of gynandromorphs will not apply to this case. There is no way to decide between the first two hypotheses, but, as I have shown elsewhere,[2] the hypothesis of chromosomal dislocation will cover all cases of gynandromorphs in *Drosophila*, while that of double fertilization will not apply to one case that gives, for itself at least, a crucial test of the alternative hypotheses. The hypothesis of chromosomal dislocation is, therefore, to be generally preferred, unless in some special case it can be shown that

[2] "Mosaics and Gynandromorphs in *Drosophila*," *Proc. Soc. Exp. Biol. and Med.*, XI, 1914.

double fertilization has actually brought about the particular results that that case shows.

Incidentally this sex mosaic (gynandromorph) and others of its kind confirm the conclusions drawn from grafting experiments in insects, namely, those in which the testes were grafted into the female and the ovary into the male without influence on the secondary sexual characters that developed later. These characters in the insects must be determined by the chromosomal composition of the cells, and not be affected by the sex "glands" as such. In contrast to this situation in the insects we find in birds that the sex "glands" of the female play an important rôle in the suppressing in the female of *some* of the secondary sexual characters—characters that appear only in the males or in castrated females. Gynandromorphs are exceedingly rare in birds, but there are a few well-authenticated cases. It is difficult to explain their occurrence under the conditions named above. It is just possible, however, that their occurrence may be accounted for in the following way. If a mosaic condition of the chromosomal complex should arise the secondary sexual characters would still all be like those of the female, owing to the presence of the ovarian secretion, but, if, in such a case, the ovary should become infected, or degenerate through senile changes, the true male parts might *sooner* develop the characteristics of the male than do the true female parts, *i. e.*, those parts of the body that have the female sex complex. This suggestion has no value unless it may lead some one to examine the condition of the ovary, when such a sex mosaic again appears.

An examination of the ovaries of many rudimentary females was made. In the majority of cases the ovaries become nearly as large as those in the normal female, and while they may contain full-sized eggs most of the eggs remain immature. Examination of the food shows that very few eggs are laid; in fact, most females lay no eggs. Of those laid some at least hatch. From these observations, and from the experiments, it seems clear that the

infertility of the rudimentary females is due, largely at any rate, to their retention of their eggs, even after copulation; and since in a few cases rudimentary females and males bred together have produced daughters as well as sons, the hypothesis of prematuration that I suggested in 1912 is not the correct explanation of the sterility of the females of the rudimentary winged stock mated to rudimentary males. Moreover, since many of the females tested, especially in the later experiments, were F_2's extracted through other fertile stocks, the sterility can not be supposed to be due to any additional peculiarity that has appeared in the rudimentary stock, but must be one of the attributes of the factor for rudimentary itself.

NOTES AND LITERATURE

DIPTERA FROM THE SEYCHELLES.—An important work has just come to hand[1] in which Mr. C. G. Lamb describes the muscoid flies of several groups, collected in 1905 in the Seychelles and other islands of the Indian Ocean by the expedition under the leadership of Mr. J. Stanley Gardiner. Considering the remoteness of the regions explored, and the total lack of general interest in the taxonomy of these small Diptera, one would not at first see any reason for calling special attention to this paper. When, however, we think of the wonderful results attained by Morgan and those working with him through the intensive study of *Drosophila,* Mr. Lamb's work, dealing in part with this very group, gains new significance and suggests many strains of thought. Some years ago, after looking at a number of the strange *Drosophila* mutants in Professor Morgan's laboratory, I raised the obvious question: "How do you know that all this has anything to do with the evolution of species?", and Professor Morgan replied that he did not know it. The real answer to my question must be found by investigating the allied species, to see whether they do in fact differ in ways at all paralleled by Morgan's mutants, or likely to have arisen in similar fashion. The important cytological paper by Metz, just published, represents one way of attacking this problem; the taxonomic results of Lamb, based on twenty species, afford us another.

At the outset, we are struck by the fact that the Seychelles *Drosophila* species have been modified in a great variety of different ways, in several cases so remarkably that Lamb hesitates whether to base new generic or subgeneric names upon them. There is nothing like orthogenesis, apparently. Here is a list of some of the more noticeable modifications:

1. Costa extending to third vein.
2. Remarkably constricted waist and short wings. (Compare Morgan's short winged forms.)
3. A remarkable slit on the costa, the end provided with spines and bristles.
4. Remarkable transverse or oblique eyes.
5. Remarkable spines on front tarsus.

[1] *Trans. Linnean Soc. London, Zoology,* Vol. XVI, Part 4, July, 1914.

6. Curious curled hairs on front legs.
7. Marmorated thorax.
8. Entirely black, except brownish antennæ and lighter face.

All the species are new except two, one of these being the *D. ampelophila* of Morgan's experiments, the prior name for which is *D. melanogaster* Meigen. One of the new species, *D. similis*, is based on males differing from *D. melanogaster* in lacking the large combs on the front legs, having instead only minute combs which require a high magnification to be seen. In other respects the flies are almost exactly as in *melanogaster*, and in the female sex it is practically impossible to distinguish between the species. There are in addition five females resembling *melanogaster* and *similis*, but differing in a detail of the venation. May we not suppose that *D. melanogaster* was introduced into the Seychelles by man and that *D. similis* and the females (left unnamed) with peculiar venation have arisen from it by mutation since that time?

T. D. A. COCKERELL

STERILITY IN A SPECIES CROSS

PROFESSOR J. A. DETLEFSEN, of the University of Illinois, has recently published an interesting paper entitled "Genetic Studies on a Cavy Species Cross."[1] Several wild cavies from Brazil (*Cavia rufescens*) were crossed with domestic guinea-pigs (*C. porcellus*) and a study of the hybrid offspring was continued for seven generations. The experiments were begun in 1903 by Professor Castle who turned them over in 1909 to Professor Detlefsen. They were carried on at Harvard and at the Bussey Institution. The paper is divided into three parts; the first two treat respectively of the genetics of color and coat factors, and growth and morphological characters; the third and most important deals with a study of the sterility of hybrids.

Cavia rufescens differs from the guinea-pig in several characters. In size it is about half as large as the guinea-pig. It has an agouti (gray) coat, but the animal has a darker appearance than tame agouti guinea-pigs, owing to the yellow bands in the ticked hair being much reduced. The belly of the wild species varies from a light yellow to a slightly ticked condition. In agouti guinea-pigs the belly hair is usually yellow with a dark base, but never ticked.

Of the wild stock, only males were used in crossing, on account of the difference in size. All F_1 males from such crosses were

[1] Carnegie Inst. Publication No. 205, 1914.

found to be sterile but the F_1 females were fertile, and the line was continued only by crossing these females with guinea-pig males. Thus with each succeeding generation there was a reduction in the amount of wild blood and the author refers to his hybrids as one half wild, or F_1, one fourth wild or F_2, etc.

As to the inheritance of coat color, the tame agouti coat is dominant over the wild agouti. These two types segregate and are allelomorphic to each other. Each is also allelomorphic to its absence. Detlefsen finds that there is a constant relation between back color and belly color, but this condition is not due to separate factors because they can not be transmitted independently. The two types of tame and wild agouti, he thinks, are perhaps comparable with the types of gray mice described by Cuénot and Morgan, viz., gray-bellied and light- (or white-) bellied agouti. In crosses with non-agouti, the wild agouti type is dominant over black and over red, as in domestic guinea-pigs. After back-crossing the wild agouti colored hybrids with non-agouti for several generations, it is found that the agouti factor is modified, producing a darker coat, in some cases almost black, the ticking being faintly seen only on the belly. Roughness of coat is imperfectly dominant over smooth coat. In later generations it regains its dominance.

In respect to growth and vigor, the F_1 hybrids were heavier at all ages than the guinea-pig parents, and were more vigorous than either parent. These F_1 individuals were crossed with guinea-pigs and gave young which were smaller than the F_1 animals in every way, and in size resembled the guinea-pig parent. The variability of the wild stock in weight and vigor is unknown, but guinea-pigs are remarkably uniform in both respects. In morphological characters, the M-shaped nasal-frontal suture of the wild species is dominant over the truncated nasal suture of the domestic form. The truncated suture reappears in the second generation but does not breed true. As to skull shape, the wild has a pointed head and the tame species a round one. In F_1 a blending occurs, and in later generations the wild pointed head disappears. In the wild cavy a narrow indenture is present on the outer surface of the last upper molar—a character held to be of much importance by systematists. In F_1 this indenture showed to a slight degree, and in subsequent generations was lost.

The section of the paper dealing with sterility is of great interest. No previous investigations on sterility in animals have

been made on such a scale as the experiments reported by Detlefsen. The causes of sterility are very obscure and but little understood. A change of environment and consequent lack of exercise or difference of diet may be a contributing cause of sterility in birds and wild animals in captivity, but none of these influences were operative in Detlefsen's experiments, because the wild cavies from Brazil bred *inter se* under laboratory conditions. Sterility is frequent in hybrids of species not closely related, and it is an axiom among biologists that crosses between different species or genera produce sterile hybrids in one or both sexes. As stated above, all F_1 hybrid males from a cross between a wild cavy male and a guinea-pig female were sterile. However, the F_1 females were fertile and were crossed back to guinea-pig males. These likewise gave in F_2 sterile males and fertile females. Repeated back crosses of fertile females with guinea-pig males produced fertile males in increasing numbers with each generation.

In order to test the fertility of the hybrid males two methods were used: (*a*) breeding tests; (*b*) microscopic examination of spermatozoa obtained by transecting several tubules from the epididymis on one side of the animal. Such an individual could be bred subsequently. In all, 483 males were tested by one or both methods; 50 by breeding alone; 331 by microscopic examination alone; and 102 by both methods. The following table, giving the results of combined microscopic and breeding tests, indicates the value of microscopic examinations in determining the fertility of males:

Microscopic Test	No.	Breeding Test	
		Sterile	Fertile
Without spermatozoa	23	23	
With immotile sperm	11	11	
With few motile sperm	10	9	1
With many motile sperm	58	14	44
	102	57	45

It will be noted that of the 58 males with many motile spermatozoa, 14 proved to be sterile upon breeding. Among these males, Detlefsen attributes the sterility of 9 to external causes, without specifying them, but he can assign no reason for the impotency of the remaining 5. He therefore concludes that

the number and motility of the sperm are not the only essentials for a real fertility, inasmuch as real fertility in the last analysis may mean

the capacity to fertilize eggs and sire young. There are further reasons for concluding that the motile sperm of the hybrid males may be physiologically different from those of the normal guinea-pig, for it often required much more time to obtain young from the hybrid males and the litters were unexpectedly small.

It may be added that sterility was not due to the absence of secondary sexual characters, for all the males were normal in this respect.

The percentage of fertile males in each generation from the back crosses above described, was as follows:

F_1	F_2	F_3	F_4	F_5	F_6	F_7
1/2 Wild	1/4 Wild	1/8 Wild	1/16 Wild	1/32 Wild	1/64 Wild	1/128 Wild
0.0	0.0	14.29	33.32	60.67	69.39	73.33

In the generations having the more dilute wild blood the percentage of fertile males increased. The author holds that some disturbance occurred in the gametogenesis of the males, subsequent to hybridization. The females were normal, but they transmitted this disturbing element to their sons. However, by back-crossing with guinea-pigs this peculiar quality was segregated out. It is evident that if the heredity of fertility and sterility in this case is Mendelian, it is due not to one or two allelomorphic pairs of factors, but to multiple factors. A table is given of the percentages of ultimate recessives expected in back crosses on the basis of various numbers of factors involved. The series for 8 factors, given below, approaches most nearly the percentage of fertile males obtained (see above):

F_1	F_2	F_3	F_4	F_5	F_6	F_7
0.0	0.39	10.1	34.36	59.67	77.58	88.16

The application is somewhat misleading, as the author states, since the probable errors are not given. In each generation the probable error would have to be calculated on the supposition that the females of the preceding generation were normally distributed, otherwise one would have to take into account the error of all the preceding generations. It is improbable that the females of any generation, except F_1, were normally distributed.

He concludes that fertility acts as a very complex recessive character, the results being in accord with the expectations if a number of dominant factors for sterility were present. After these dominant factors were eliminated, there would be produced a fertile recessive type.

Byron B. Horton

FLOWER PIGMENTS

Recent researches by Wheldale and Bassett[1] have shown that there are four flower pigments, *i. e.*, ivory, yellow, red and magenta, in *Antirrhinum majus*, and that these, in various combinations and in different states of concentration and dilution, are responsible for all the color varieties. The ivory and yellow pigments have been identified with apigenin and luteolin respectively, *i. e.*, members of the class of soluble yellow plant pigments containing carbon, hydrogen and oxygen; the red and magenta pigments are anthocyanins. The yellow, red and magenta pigments occur only in the epidermis of the corolla, but ivory is present in the inner tissues. The pigments are present in the plant as glucosides, that is combined with sugar. For preparation, the flowers are boiled with water, the pigments precipitated as insoluble lead salts from the filtered solution by adding lead acetate. The lead salts are filtered off and decomposed with dilute sulphuric acid which forms insoluble lead sulphate and sets free the pigment again in dilute acid solution. These solutions are then boiled for several hours, whereby the sugar is split off from the glucoside and the free pigment, which is less soluble, separates out and is filtered off. The anthocyanins are separated from the yellow (flavone) pigments by extracting the latter with ether in which the anthocyanins are insoluble. The red and magenta pigments have been purified and analyzed and shown to contain carbon, hydrogen and oxygen only but a higher percentage of oxygen than the flavones. Determination of the molecular weights of the anthocyanins also indicates that their molecules are larger than those of the flavone pigments. Hence if the anthocyanins are derived from the flavones, it seems likely that the process is one of oxidation accompanied by condensation of two or more flavone molecules or the union of flavone molecules with other allied compounds in the plant. It is possible that the factors for red and magenta color will come to be expressed in terms of chemical substances which condense with the flavones to from the larger molecules of the anthocyanins.

M. W.

[1] Wheldale, M., "The Flower Pigments of *Antirrhinum majus*. 1. Method of Preparation," *Biochem. Jour.*, 1913, 7, 87. Wheldale, M., and Bassett, H. Ll., "The Flower Pigments of *Antirrhinum majus*. 2. The Pale Yellow or Ivory Pigment," *Biochem. Jour.*, 1913, 7, 441; "The Chemical Interpretation of Some Mendelian Factors for Flower-color," *Proc. Roy. Soc.*, 1914, B, 87, 300; "The Flower Pigments of *Antirrhinum majus*. 3. The Red and Magenta Pigments," *Biochem. Jour.*, 1914, 8, 204.

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ON THE NATURE OF THE CONDITIONS WHICH DETERMINE OR PREVENT THE ENTRANCE OF THE SPERMATOZOON INTO THE EGG

JACQUES LOEB

The Rockefeller Institute for Medical Research, New York

I

The well-known fact that a spermatozoon can no longer enter an egg after it is once fertilized raises the question whether this is due to the changes necessarily connected with development; or whether development of an egg can take place without the existence of such a block. We are in possession of facts speaking in favor of the second view. Thus the writer has shown that if the eggs of *Strongylocentrotus purpuratus* or *Arbacia* are induced to develop by the methods of artificial parthenogenesis a spermatozoon can enter the egg or an individual blastomere of a segmenting egg, while the latter is in the full process of development. This leaves no doubt that the block caused by the entrance of a spermatozoon into an egg for the entrance of further spermatozoa must be due to a change not necessarily identical with that inducing the development of the egg.

A second group of observations made by the author deals with the phenomena of specificity and these prove that the block which an egg offers to heterogeneous sperm is rapidly reversible and confined to the surface of the egg or the spermatozoon or both. In the case of the egg of *purpuratus* and sperm of *Asterias* (and many similar in-

stances) the specific block can be overcome if we slightly increase the alkalinity of the sea water. The spermatozoon can only enter the foreign egg while both sperm and egg are in the hyperalkaline sea water, whereas if the egg and sperm are treated separately with hyperalkaline solution (no matter how long) and put together in a sufficiently large quantity of normal sea water no egg can be fertilized[1] while fertilization will take place as soon as the hyperalkalinity is restored. This shows that the change (brought about by the hyperalkaline sea water) which makes the fertilization possible is rapidly reversible, as we should expect it to be if it consisted merely in a physical change at the surface of the egg. To this series of facts, others might be added which point in the same direction. In this paper we intend to discuss a little more fully the various conditions which block or favor the entrance of a spermatozoon into an egg, in order to form an idea of the nature of the forces which control these phenomena.

II

1. When the unfertilized eggs of *S. purpuratus* are treated for two hours with hypertonic sea water (50 c.c. sea water + 8 c.c. $2\frac{1}{2}$ *m* NaCl or Ringer solution) the eggs of certain females will develop into blastulæ, gastrulæ and plutei, while the eggs of other females can not be caused to develop in this way. These individual differences coincide possibly with those observed by the writer in regard to spontaneous membrane formation in the eggs of different females[2] and it is possible that only the eggs of such females of *purpuratus* can be induced to form larvæ through a mere treatment with hypertonic sea water in which the latter can induce the cortical changes underlying the membrane formation. Whatever the nature of the individual difference may be, *purpuratus* eggs

[1] The large quantity of sea water is necessary so that the hyperalkaline sea water at the surface of the egg and sperm can diffuse away before both gametes come in contact.

[2] Loeb, *Arch. f. Entwcklngsmech.*, XXXVI, 626, 1913; "Artificial Parthenogenesis and Fertilization," Chicago, 1913, p. 219.

which have been induced to develop into larvæ by a hypertonic solution can be fertilized with sperm while they are in the process of segmentation. When such eggs are in the two-, four-, eight-, or sixteen-cell (and possibly also later) stages the sperm can enter into one or more blastomeres of such an egg and this entrance betrays itself by a distinct and clear membrane formation around each blastomere.[3] While the segmenting eggs which were not fertilized with sperm develop into larvæ, those into which sperm enters perish very rapidly. This simple and rather striking experiment which can easily be performed in the eggs of *Strongylocentrotus*, where the membrane formation around a single blastomere can be clearly recognized, shows that the process of development in a fertilized egg in itself can not be responsible for the block caused by fertilization. It looks as if the entrance of a spermatozoon into the mature egg, independently of the developmental changes it induces in the egg, causes some physical or physico-chemical change (of the surface of the egg?) which renders the subsequent entrance of a spermatozoon impossible.

2. With the eggs of most females of *purpuratus* the treatment with a hypertonic solution does not lead to a development into larvæ, but only to the first segmentation stages in a limited number of eggs (provided that the eggs have been exposed to the solution the proper period of time). Such blastomeres afterwards go into a resting stage. If one waits long enough, until there is no doubt left that the blastomeres have reached a resting condition and will divide no further, and if one then adds sperm, the individual blastomeres can again be fertilized, which is indicated by a membrane formation around each individual blastomere and the subsequent development of such blastomeres into swimming larvæ.[4] The fact that each individual blastomere in this case is fertilized independ-

[3] Loeb, "Artificial Parthenogenesis and Fertilization," p. 240; *Arch. f. Entwcklngsmech.*, XXIII, 479, 1907.

[4] Loeb, *Arch. f. Entwcklngsmech.*, XXIII, 479, 1907; "Artificial Parthenogenesis and Fertilization," p. 237.

ently of its neighbors suggests that there is no protoplasmic connection between the neighboring blastomeres; otherwise the entrance of a spermatozoon into one should cause its neighbors also to form a fertilization membrane, which does not happen.

All these facts show that the changes underlying development do not necessarily prevent the entrance of a spermatozoon into an egg fertilized by sperm.

3. Development can be initiated in an unfertilized egg by causing a membrane formation by a fatty acid. Eggs after such an artificial membrane formation perish as a rule rapidly at room temperature (if no second treatment is given them) but they may segment if kept at a low temperature. The eggs are usually put after treatment with the butyric acid into normal sea water in which they form a membrane. This membrane is different in the eggs of different species of sea urchins. In the egg of *S. purpuratus* the membrane is tough and entirely impermeable to the spermatozoon. When we add sperm to such eggs with a butyric acid membrane they behave exactly as if no sperm had been added, they all perish rapidly (at room temperature). The question arose, if a spermatozoon could still enter the egg of *purpuratus* after membrane formation, provided the membrane could be destroyed. This can be done in a certain percentage of the eggs of *purpuratus* by shaking them after artificial membrane formation; the number of eggs whose membrane is torn varies in different experiments owing probably to differences in the thickness and toughness of the membrane. Even if the membrane is torn the edges may come close together again so that the opening often is closed again and no spermatozoon can go through. Kupelwieser and the writer performed this experiment on the eggs of *purpuratus* and it was found that such eggs with torn membranes were fertilized upon the addition of sperm and developed normally; while the eggs whose membranes were intact all perished.[5]

[5] Loeb, "Artificial Parthenogenesis and Fertilization," p. 234.

The writer repeated this experiment last winter with the same result. He found that eggs with torn membranes when subsequently fertilized with sperm did not form any new membranes as he had stated before. It is possible that he mistook at that time the new hyaline membrane which forms around the egg after membrane formation and fertilization for a new fertilization membrane.

It is not necessary that these eggs be fertilized immediately after the artificial membrane formation, the experiment succeeds also after some time (one hour or more); only with this difference that the eggs perish very rapidly after the membrane formation if they receive no second treatment. In order to avoid this difficulty the writer last winter proceeded as follows: Artificial membrane formation was produced in the eggs of a *purpuratus* and all eggs had formed perfect membranes. One control was kept and the rest were shaken. These were divided into three lots, one served as a control; the eggs of the latter all perished as fast as the eggs of the first control (which were not shaken). The second lot were fertilized after about one half hour after membrane formation. Twenty per cent. of these eggs developed into normal larvæ, the rest perished. The percentage of developing eggs corresponded roughly with the percentage of eggs whose membrane was torn. The third lot of the shaken eggs was put overnight into 50 c.c. sea water + 7 drops of 1/10 per cent. KCN, to prevent the disintegration of these eggs. The next morning (sixteen hours after the membrane formation) the eggs of Lot 3 were transferred into normal sea water and divided into two lots, one was fertilized with sperm, the other was kept as a control. About twenty per cent. of the eggs which were fertilized began to segment, but many in an abnormal way and none developed into larvæ. Of the second lot to which no sperm was added also a few began to segment. As the writer has shown in former experiments, the eggs of *Strongylocentrotus* can be caused to develop after artifi-

cial membrane formation if they are either treated for a short time with a hypertonic solution or if for a longer period the oxidations are suppressed in them by lack of oxygen or the addition of cyanide. There is therefore no doubt that the eggs of *purpuratus* in which the artificial membrane formation has been induced by butyric acid can be fertilized subsequently with sperm.

4. The treatment of the eggs of *Arbacia* with butyric acid leads to the formation of a membrane which varies considerably in the eggs of the same female. Some eggs have a thin membrane which is permeable to the spermatozoon, others have a tough fertilization membrane which is as impervious to the spermatozoon as the regular fertilization membrane. The percentage of the eggs with membranes permeable for sperm varies very much in different experiments, according to the material and according to the external conditions. If this is kept in mind it is easily understood that the number of *Arbacia* eggs which can be fertilized after they have been treated with butyric acid differs in different experiments. Since the membrane called forth by butyric acid is not always plainly visible, it is a prerequisite that always one set of such eggs should be set aside as controls to ascertain whether or not *all* the eggs disintegrate rapidly (if no second treatment is given to them). Only if they all disintegrate rapidly have we any guarantee that in all of them the membrane formation has been effective. The former experiments of the writer show that such eggs can be fertilized by sperm; in fact they show that while the unfertilized eggs disintegrate rapidly after the inducement of the membrane formation with butyric acid, the subsequent fertilization of such eggs by sperm saves their lives and makes them develop.[6]

III

1. It is a well-known fact that most eggs can only be fertilized by sperm of their own or a closely related species. The writer thought that in order to obtain light

[6] Loeb, *Arch. f. Entwcklngsmech.*, XXXVIII, 416, 1914.

on the nature of the block to the entrance of heterogeneous sperm it was necessary first to find the means by which this block could be overcome. He succeeded in showing that the egg of the sea urchin *S. purpuratus* can be fertilized by the sperm of starfish, brittle stars, and holothurians in sea water (or other balanced solutions) if their alkalinity was a trifle higher than that of ordinary sea water (*e. g.*, in a solution of 50 c.c. sea water + 0.6 c.c. $N/10$ NaOH).[7] Godlewski[8] succeeded by the same method in the fertilization of the egg of the sea urchin with the sperm of crinoids

The most important fact found out in this connection was the following, namely, that the fertilization of the egg of *purpuratus* by the sperm of *Asterias* only takes place while both eggs and sperm are in this hyperalkaline solution. If eggs and sperm are put into these solutions separately and if then from time to time sperm and eggs so treated are transferred into normal sea water, as a rule not a single egg is fertilized; while with the same material when eggs and sperm are together in the hyperalkaline solution as many as 100 per cent. of the eggs may be fertilized. The effect of the alkali is, therefore, rapidly reversible; the eggs when put from the hyperalkaline sea water free from sperm into the normal sea water containing very motile sperm of *Asterias* can not be fertilized; when put back into hyperalkaline sea water containing *Asterias* sperm they will be fertilized rapidly.

This rapid reversibility of the effect of the NaOH indicates that it must be confined to the surface of the egg and the spermatozoon or both; and this is corroborated by the fact that the NaOH does not enter the cells. One of the forces which determine the entrance of the spermatozoon into the egg may be surface tension and the phenomenon of the entrance may be comparable or possibly identical with the phenomenon of phagocytosis.

Godlewski mentioned that he occasionally observed a

[7] Loeb, *Pflüger's Arch.*, IC, 323, 1903; CIV, 325, 1904; *Arch. f. Entwcklngsmech.*, XL, 310, 1914; *Science*, N. S., XL, 316, 1914.

[8] Godlewski, *Arch. f. Entwcklngsmech.*, XX, 579, 1906.

fertilization of the egg of the sea urchin with the sperm of a crinoid in normal sea water after both had been treated with hyperalkaline sea water separately. This observation is correct but finds its explanation in the assumption that in such cases the hyperalkaline sea water had not had time to diffuse from the jelly of the egg or from the surface of the egg protoplasm by the time the spermatozoon came in contact with it. In order to test this view the writer treated the eggs of *purpuratus* with a hyperalkaline solution of greater than the optimal concentration while the sperm was treated separately with the optimal concentration (50 c.c. sea water + 0.6 c.c. $N/10$ NaOH) and then both were mixed in a little sea water in a watch glass. In such a case a large number of eggs were fertilized, but while the fertilization occurred nominally in normal sea water it really occurred in a layer of hyperalkaline sea water surrounding the protoplasm of the egg.

The conclusion from these experiments is that the block to the entrance of the spermatozoon of *Asterias* into the egg of *purpuratus* is of a rapidly reversible character, consisting in some alteration of a physical property of the surface. On this assumption the factor of specificity consists of an agency which affects these properties of the surface of the egg in the same sense as the increase in the concentration of the alkali. It should be added that the writer observed also that an increase of the concentration of Ca in the sea water acts in the same sense as an increase in the alkalinity; and that if the concentration of Ca is increased the increase of NaOH may be less than is necessary otherwise.

2. If the idea was correct that the factor of specificity contained in the spermatozoon affected only the forces acting at the surface of the egg; and that the lack of this factor could be replaced by a rise in the alkalinity of the sea water, it was to be expected that the reverse should also be possible: namely, that a change in alkalinity or the constitution of the surrounding medium should pro-

duce a reversible block to the spermatozoa of the same species. That means, it should be possible to find solutions in which the egg does not suffer for a long time, in which the sperm lives for a long time, and in which the sperm of the same species is intensely active and attacks the egg with the greatest eagerness and yet is not able to enter; while if the medium is but slightly changed the sperm enters the egg at once. The writer carried out such experiments a year ago in Pacific Grove and last summer in Woods Hole and found this to be true.[9]

For the purpose of these experiments the ovaries and testes of the sea urchins were not put into sea water but into a pure $m/2$ NaCl solution (after several washings in such a solution) and kept in such a solution. Several drops of sperm and one drop of eggs were in one experiment put into 2.5 c.c. of a neutral mixture of $m/2$ NaCl and 3/8 m $MgCl_2$ in the proportion in which these two salts exist in the sea water. In such a neutral solution no egg of *Arbacia* or of *purpuratus* is fertilized no matter how long they remain in the solution, although the sperm is very active. If the eggs and sperm are transferred into the same solution which contains in addition 1 drop of a $N/100$ solution of NaOH (or NH_3, or benzylamine, or butylamine) or 8 drops of $m/100$ $NaHCO_3$, most and often practically all the eggs at once form fertilization membranes and begin to segment at the proper time.

The same result can be obtained if the eggs are transferred into a neutral mixture of $NaCl + MgCl_2 + CaCl_2$ (in the proportion in which these salts exist in the sea water) or into a neutral mixture of $NaCl + MgCl_2 + CaCl_2 + KCl$. In such a neutral mixture the eggs form fertilization membranes and begin to segment.

The eggs will not be fertilized if transferred into a neutral solution of NaCl or of $NaCl + KCl$.

It is, therefore, obvious that if we diminish the alkalinity of the solution surrounding the egg and if we deprive this solution of $CaCl_2$ we establish the same reversible

[9] Loeb, *Science*, N. S., XL, 316, 1914.

block to the entrance of the spermatozoon of *Arbacia* into the egg of the same species as exists for the entrance of the sperm of starfish into the egg of *purpuratus* in normal sea water.

Another form of the experiment may be mentioned. When we put sperm and eggs of *Arbacia* (which had been washed in an $m/2$ NaCl solution) into a neutral mixture of NaCl + KCl no egg can be fertilized although the sperm may be so active and concentrated that the eggs roll around in the solution and the chorion (the jelly surrounding the egg) may be filled with spermatozoa. In one experiment the eggs and sperm of *Arbacia* were kept overnight in watch glasses containing 2.5 c.c. of this mixture of neutral NaCl + KCl. The next morning all the eggs were intact and not a single one was fertilized. At that time 20 drops of sea water were added to the mixture and instantly fertilization membranes were formed and practically all the eggs segmented.[10]

It can be shown that in this experiment the sea water added two important substances, Ca and NaOH. If NaOH alone is added to the mixture of NaCl + KCl, as a rule no egg is fertilized or only a few; if $CaCl_2$ is added to a neutral mixture of NaCl + KCl a number of eggs are fertilized. If both $CaCl_2$ and NaOH are added in the proper proportion as a rule all the eggs are fertilized.

It is perhaps important to call attention to the fact that if eggs of *Arbacia* are fertilized in sea water and if after repeated washings in a mixture of NaCl + KCl or of NaCl + $MgCl_2$ they are put into these solutions they will segment repeatedly in these solutions, thus showing that the eggs were really not fertilized in these two solutions in the above-mentioned experiments.

The striking fact is again that the block created by the

[10] This experiment was carried out with different concentrations of sperm and it was found that only in the dishes where the concentration of sperm was sufficiently high were all the eggs fertilized upon the addition of sea water. This is perfectly natural as the majority of spermatozoa die gradually (as do also the eggs) and hence enough spermatozoa will only be alive the next day if the concentration of sperm was not too low.

lack of $CaCl_2$ or NaOH or both to the entrance of the spermatozoon is removed immediately after these substances are added. The block must be due merely to a change in the physical condition of the surface (which may be based on a rapidly reversible chemical reaction).

In these experiments the NaCl can not be replaced by isotonic sugar solutions. The same fact was found by the writer to be true for heterogeneous hybridization.

It is of importance to call attention to the fact that the abolition of the block in the case of heterogeneous hybridization depends upon the same substances, $CaCl_2$ and NaOH (or some other alkali), which make normal fertilization possible. The influence of electrolytes on the fertilization of the egg of *purpuratus* by the sperm of *Asterias* is parallel to the influence of the same electrolytes on the fertilization of the same egg by the sperm of *purpuratus;* only the concentrations differ, and always in the same sense. The forces at work are, therefore, apparently the same in both cases; but we can only express surmises as to their nature. The rôle of salts as well as the rapid reversibility indicate that they are forces situated at the surface of the egg and the spermatozoon or both. In the first place we may think of surface tension conditions and in this respect it is possible that the entrance of the spermatozoon into the egg may be determined by such forces in a way similar to the process of phagocytosis. In the second place it may be that previous to the action of surface tension forces an alteration in the degree of fluidity of the egg surface may be required (*e. g.*, that physical change which finds its expression in the formation of the fertilization cone). Thirdly, it may be possible that before the surface tension forces can act the spermatozoon must agglutinate with the egg surface and that this agglutination is determined by certain specific substances or by certain salts ($CaCl_2$ and NaOH) or by both.

Brief mention should be made of the block discovered by Godlewski[11] to the entrance of a spermatozoon into

the egg if the sperm of the same species is mixed with the sperm or the blood of a species widely apart. If, for instance, the sperm of a sea urchin is mixed with the sperm of certain annelids (*Chætopterus*) or molluscs and if after some time the eggs of the same sea urchin are added to the mixture of the two kinds of sperm no egg is fertilized. If the solution is, however, subsequently diluted with sea water or if the egg that was in this mixture is washed in sea water, the same sperm mixture in which the egg previously remained unfertilized will now fertilize the egg. From these and similar observations Herlant[12] draws the conclusion that the block existed at the surface of the egg, inasmuch as a reaction product of the two types of sperm is formed after some time which alters the surface of the egg and thereby prevents the sperm from entering. This view is not only supported by all the experiments but also by the observation of the writer that foreign sperm or blood is able to cause after some time a real agglutination if mixed with the sperm of a sea urchin or a starfish.[13] We can imagine that the precipitate forms a film around the egg and acts as a block which can be removed mechanically by washing.

It is not impossible that the block which exists in the fertilized egg is due also to an alteration of the physical character of the surface of the egg which in this case is, however, induced from within the egg by changes caused by the entrance of the spermatozoon, which, however, are not necessarily identical with those causing development as was shown by the facts in the second chapter.

IV.

We will now turn to the question whether the motility of the spermatozoon plays no other rôle than to bring the spermatozoon so close to the surface of the egg that surface tension phenomena can engulf the spermatozoon into the egg. It is easy to show that if the spermatozoa

[11] Godlewski, *Arch. f. Entwcklngsmech.*, XXXIII, 196, 1911.

[12] Herlant, *Anat. Anzeiger*, XLII, 563, 1912.

[13] Loeb, *Jour. Exper. Zool.*, XVII, 123, 1914.

of *purpuratus* are immobilized by NaCN no egg of the same species can be fertilized, no matter how concentrated the sperm; while the same sperm when it revives from the effect of NaCN fertilizes the same eggs at once. This meets with the possible objection that the motility of the sperm might be only necessary to allow the latter to penetrate the jelly surrounding the egg protoplasm. In order to test this objection the writer freed the eggs of *purpuratus* from this jelly by treating them for two minutes in a mixture of 50 c.c. sea water + 3 c.c. $N/10$ HCl in which all the jelly is dissolved. The eggs were washed afterwards in sea water and it was found that if sperm was added practically all were fertilized. The writer put such eggs with sperm which was immobilized by NaCN. The eggs and the sperm were squirted together with a pipette in order to bring about a close contact. No matter how concentrated the sperm was, not a single egg was ever fertilized. As soon as the spermatozoa recovered and showed only a slight degree of motility fertilization became possible. This leaves no doubt that the motility of the sperm is one of the forces required to bring the spermatozoon into the egg.

That motility is not the only force was already indicated by the previous chapter which made it clear that even if the sperm is active it can not enter the egg unless certain physical conditions at the phase boundaries of egg, spermatozoon and surrounding solution were right. In order to leave no doubt about this fact the following experiments may be quoted. If we put NaCl sperm[14] of *purpuratus* or of *Arbacia* into a neutral mixture of NaCl + KCl containing eggs of the same species the sperm will sooner or later become very active. Yet not a single egg is fertilized. If we make the solution slightly alkaline the sperm becomes at once extremely active yet with a few exceptions no egg is fertilized; while much less active sperm will fertilize all the eggs if $CaCl_2$ is added. The second fact is this: that the most active

[14] Sperm from testicles washed in $m/2$ NaCl and kept in such a solution.

sperm of *Asterias* will not fertilize the eggs of *purpuratus* in sea water while it will do so in hyperalkaline sea water (50 c.c. sea water + 0.6 c.c. $N/10$ NaOH).

We, therefore, arrive at the conclusion that aside from the physical conditions at the surface of the egg and the spermatozoon the impact of the spermatozoon against the egg is a prerequisite for the process of fertilization.

von Dungern was, as far as the writer is aware, the first to call attention to the fact that the egg itself causes resting spermatozoa to become active,[15] but curiously enough he tried to show that only foreign sperm is "stimulated" in this way by the egg (which is, as F. Lillie pointed out, not correct) and v. Dungern tried to explain on this basis why it was not possible to fertilize the egg of the sea urchin with the sperm of the starfish which had at that time not yet been accomplished.

von Dungern noticed that the egg of the sea urchin "stimulates" the spermatozoon of starfish to greater action and he concluded that since "stimulation" according to Jennings causes "motor reaction" whereby the direction of the motile organism is changed this very stimulating influence of the egg of the sea urchin upon the spermatozoon of the starfish prohibited the latter from getting into the egg. On the basis of the same idea von Dungern was consistently led to the further conclusion that the egg exercised no "stimulating" influence upon spermatozoa of its own species and that thereby the spermatozoon of the same species was enabled to get into the egg. A year after the appearance of von Dungern's paper the writer succeeded in accomplishing the hybridization of the sea urchin egg with starfish sperm by a method which contradicted von Dungern's theory, namely, by increasing the alkalinity of the sea water whereby the spermatozoon is "stimulated" to still greater activity; and on the other hand it is a common experience that a sea urchin spermatozoon becomes more active when it comes near an egg of its own species.

The writer was anxious to compare the activating

[15] v. Dungern, *Ztsch. f. allg. Physiol.*, I, 34, 1902.

action of eggs of the same and various foreign species upon spermatozoa. Since the spermatozoa of the sea urchins are usually very active in pure sea water (*i. e.*, sea water free from egg substance) it was necessary to find a solution in which these spermatozoa will keep alive for a number of days without showing any motility. Such a solution was found in a neutral $m/2$ NaCl solution and this led to the method of putting ovaries and testes directly into such solutions instead of into sea water.[16] The ovaries and testes were first washed repeatedly in these solutions to free them from the blood or its salts, and then one drop of eggs and one or more drops of the sperm suspension were mixed in a watch glass containing 5 c.c. $m/2$ NaCl (free from egg contents). In one experiment the sperm and eggs of two sea urchins, *purpuratus* and *franciscanus*, and two starfish, *Asterias ochracea* and *Asterina* (at Pacific Grove), were used. None of the four forms of spermatozoa showed any motility in a pure NaCl solution (without egg contents). In sea water (free from egg contents) the spermatozoa of the two forms of sea urchins were very active, those of the starfish were immobile. The starfish eggs were immature and did not mature during the experiment (those of *Asterias* were out of season and very small); the sea urchin eggs were mature. The result is indicated in the following table.

That there exists no strict specificity is obvious by the fact that the immature eggs of *Asterina* activate the sperm of the sea urchin *franciscanus* as powerfully as is done by the mature eggs of the sea urchin *purpuratus* and *franciscanus*. But the spermatozoa of the two species of starfish show a marked specificity inasmuch as they are activated strongly only by the (immature) eggs of their own species and only to a slight degree by the

[16] The writer had found previously that the unfertilized eggs of *purpuratus* are killed more rapidly in sea water than in a neutral $m/2$ NaCl solution, probably on account of the greater alkalinity of the former. The same may be true for the sperm of this species, athough this has not yet been tested. The unfertilized egg of *Arbacia* is more sensitive to a pure NaCl solution than that of *purpuratus*.

TABLE I

Specificity of Activation of Sperm by Eggs

	Asterias ♂	*Asterina* ♂	*Franciscanus* ♂	*Purpuratus* ♂
Asterias ♀ (immature)..	*Immediately very motile.*	No activation.	Moderately active.	Slight effect in immediate contact with egg.
Asterina ♀ (immature).	Not motile.	*Violent activity.*	*Violent activity.*	Slight effect only near the egg.
Franciscanus ♀ (mature)	Slightly motile.	No motility.	*Immediately active.*	*Immediately motile.*
Purpuratus ♀ (mature).	Slightly motile after some time.	Slight effect in immediate contact with eggs.	*Immediately active.*	*Immediately active.*

eggs of the sea urchin *purpuratus*. In judging these results the reader must keep in mind first that all these experiments are made in a NaCl solution, and second, that it requires a stronger influence to activate the spermatozoa of the starfish which are at first not motile in sea water (free from egg contents) than the sea urchin spermatozoa which are from the very first very active in such sea water and which may therefore be considered as being at the threshold of activity in the pure NaCl solution.

If instead of the eggs themselves the supernatant NaCl solution from eggs is added to the sperm it is found that it requires a very much greater concentration of the supernatant NaCl solution from *Asterias* eggs to arouse the *purpuratus* sperm in NaCl into activity than if the supernatant NaCl solution from *purpuratus* or from *franciscanus* eggs is used.

The question now arises whether the relative influence of the egg on the motility of the sperm bears any relation to the power of the latter to enter the egg; or in other words if we can foretell which forms will hybridize by observing the relative activating effect of the eggs upon the spermatozoa. This does not appear to be the case on the basis of our present limited experience, since the activating effect of the *franciscanus* egg upon the sperm of *Asterias* is just as great if not greater than that of *purpuratus* eggs and yet *Asterias* sperm can enter the

latter and not the former. Even if we intensify the activity of the spermatozoon of *Asterias* by putting it in hyperalkaline sea water it will not enter the egg of *franciscanus.*

If we mix eggs of *franciscanus* and *purpuratus* in sea water and add the sperm of *purpuratus* the eggs of *purpuratus* will be fertilized more quickly than the eggs of *franciscanus;* and the reverse is true if the sperm of *franciscanus* is added to a mixture of both eggs in sea water. The writer is not quite certain that this difference is accompanied by a corresponding difference in the influence of these eggs upon the motility of their spermatozoa. It is certain, however, that the addition of egg sea water from *Asterias* does not help the fertilization of *purpuratus* eggs by *Asterias* sperm, although the egg sea water from *Asterias* increases the activity of *Asterias* sperm.

The writer is, however, of the opinion that this activating effect of the egg upon the spermatozoon is of the greatest importance for fertilization in nature and that the degree of specificity which exists (although it is far from absolute) is a means of preventing hybridization. The writer is under the impression that the eggs which are naturally fertilized in water are fertilized almost instantly after they are shed. Thus it is stated at hatcheries that the egg of the salmon loses its power of being fertilized in a few minutes and in the case of *Fundulus* the egg loses this power also very rapidly. The ripe egg of starfish dies rapidly if not fertilized. On the other hand, the writer has often been struck with the fact that the sperm of most marine forms when put into sea water is at first practically not motile. When the eggs have a specific gravity considerably greater than the water (as is the case for *Fundulus*) the eggs will sink very rapidly while the sperm remains suspended for some time. Now we have mentioned that if the absolutely inactive sperm of *Asterias* or *Asterina* comes in contact with eggs of its own species (even if they are immature) it is at once aroused into violent activity. If

the same were true for the egg of *Fundulus* fertilization could take place probably before the egg reaches the bottom of the water. If by chance a teleost of a different species would shed its sperm in the immediate neighborhood and some of it could reach the egg of *Fundulus* while it is falling the foreign sperm could probably not be aroused as quickly by the egg of *Fundulus* as the sperm of the *Fundulus* male and hence no hybridization would occur. In fish we can see that the male and female shed their sexual cells simultaneously so that they come at once in contact. The writer is inclined to believe that something similar occurs also in Echinoderms. He had last year a chance to verify once more an observation he had made for a number of years and which he had already mentioned in a previous publication.[17] The sea urchins at Pacific Grove are found in large numbers on rocks in certain coves near the shore. Up to a certain day in March every female of *purpuratus* was full of eggs. On the next day the surface of the sea in this region showed the usual indication of the spawning of large masses of animals: namely the enormous foam formation in the little coves although the sea was only moderately agitated. This foam formation is due to an increase of organic substances which lower the surface tension of the sea water and make the foam more durable. The writer realized that this might mean the end of material for some time to come and indeed not a single female of *purpuratus* of hundreds opened on that day had eggs. The condition was the same for all the sea urchins collected for two miles along the shore. During the next week immature eggs began to appear again in the sea urchins and in about ten days ripe eggs were again found. This indicates that in this region the males and females shed their eggs and sperm simultaneously. It is not impossible that among sea urchins which are found in colonies on the rocks the shedding of the sexual products of one or several individuals acts as an incentive for the whole colony. Since the eggs fall in this case also much

[17] Loeb, "The Mechanistic Conception of Life," Chicago, 1912, p. 196.

more rapidly to the bottom than the spermatozoa it is also very probable that the eggs are fertilized before they reach the bottom of the sea. We can understand under these circumstances that the specificity which exists in the activating effect of the egg upon the sperm is one of the safeguards against hybridization for eggs that are fertilized in the water, inasmuch as this specificity activates the sperm of the same species much more quickly than that of a foreign species. Other safeguards are the phase-boundary conditions which we discussed in the previous chapter.

V

If we assume that the spermatozoon bores itself into the egg by the energy of the vibrations of its flagellum it is easy to understand the importance of its motility for this process. It is, however, equally possible that a certain energy of vibration is needed to make the spermatozoon stick to the surface of the egg and that afterwards forces of a different character bring the spermatozoon into the egg. The fact that under normal conditions a very slight degree of motility on the part of the spermatozoon allows it to enter the egg seems to favor such a view.

von Dungern had already discussed the possible rôle of phenomena of sperm agglutination in fertilization as a protective agency. F. Lillie discovered the transitory agglutination of sperm induced by a substance from eggs of the same species.[18] When the sperm of the sea urchin *Arbacia* is mixed with the supernatant sea water from eggs of the same species a cluster formation occurs which may last a number of minutes and which is essentially a transitory agglutination. In *Arbacia* the agglutination is very striking, in *purpuratus* the phenomena of agglutination are not lacking but the writer was under the impression that other phenomena of the type of tropisms might enter. But he was not very certain on this point and left that question open for further discussion. The

[18] F. Lillie, *Science*, N. S., XXXVIII, 524, 1913; *Jour. Exper. Zool.*, XVI, 523, 1914.

writer is, however, under the impression that no proof for the existence of a positive chemotropism of the sea urchin sperm for the eggs of the same species has thus far been given.

The writer observed that this phenomenon of sperm agglutination depends on the motility of the sperm:[19] It only appears when the sperm is extremely motile and it lasts only a number of minutes, often only a fraction of a minute as Lillie had found. The writer observed that the duration of the clusters depended to some extent on the alkalinity of the solution. The more alkaline the latter the more rapidly the cluster scatters. The presence of a salt with a bivalent metal, especially Ca, seems necessary for the cluster formation. Sr and Ba act like Ca and so does Mg but in the latter case a slightly higher concentration is needed. The more Ca is added the more powerful the agglutination becomes. These facts suggest the following origin of the agglutination. From the jelly surrounding the eggs a certain substance is dissolved in the sea water which reacts chemically with a certain substance at the surface of the spermatozoon. If this reaction takes place in the presence of one of the salts of a bivalent metal, especially Ca, a sticky precipitate is formed on the surface of the spermatozoa, which is slowly soluble in the solution; and the more rapidly the more alkaline the solution. If the spermatozoa are very active the impact with which they strike each other may lead to their sticking together and this agglutination will last until the precipitate is dissolved again.[20]

The writer mentions this fact here because it might give us a clue to the rôle of the motility of the spermatozoon for its entrance into the egg. One can imagine that the spermatozoon must stick to the surface of the egg in order to be taken into it and this sticking may not come about unless the spermatozoon strikes the surface of the

[19] Loeb, *Jour. Exper. Zool.*, XVII, 123, 1914.

[20] Lillie measures the degree of agglutination by its duration; if our assumption is correct he really measures the time required for the solution of the sticky precipitate on the surface of the spermatozoon by the sea water.

egg with a certain velocity. This is, however, merely a suggestion. The really serious difficulty of such an assumption lies in the fact that the specific and transitory cluster formation or agglutination of the spermatozoa is not a general phenomenon. It may even turn out to be confined to sea urchins and certain annelids. It is probably lacking in all cases of hybridization. Yet this would not necessarily speak against the possibility of an agglutination of the spermatozoon to the egg as a prerequisite of fertilization.

This latter idea receives some support in the writer's experiments on heterogeneous hybridization. He was able to show that both NaOH as well as $CaCl_2$, which render possible the fertilization of the eggs of certain sea urchins through the sperm of starfish, also favor the agglutination of that sperm to the chorion of the egg. This leads to the peculiar phenomenon of mere membrane formation in the egg by the living spermatozoon without the entrance of the latter into the egg.[21]

VI

Lillie seems to take it for granted that the substance of the egg which causes sperm agglutination is identical with the substance which stimulates the spermatozoa into greater activity. If this were correct the conditions for the two phenomena should be identical, which is however far from being the case.

The writer showed that if we deprive the eggs of *purpuratus* of the jelly which surrounds them and if we wash them afterwards a few times in sea water to deprive them of the last vestiges of jelly substance which may still adhere to them they have lost completely and permanently the power of forming clusters with the sperm of their own species. Such eggs were washed four times in $m/2$ NaCl and when a drop of the supernatant NaCl solution was added to NaCl sperm of *purpuratus* which was not motile it activated the sperm very powerfully.

The writer had found that the egg sea water of *S. fran-*

[21] Loeb, *Arch. f. Entwcklngsmech.*, XL, 310, 1914.

ciscanus does not give a trace of agglutination with the sperm of *purpuratus* but if the experiment is made in *m*/2 NaCl solutions it can be shown that the *franciscanus* egg NaCl solution activates the NaCl sperm of *purpuratus* in an *m*/2 NaCl solution very strikingly.

The immature eggs of *Asterias ochracea* activate the otherwise non-motile sperm of the same species, but the eggs of this starfish do not give any agglutination reaction with their own sperm and Lillie found the same for the starfish in Woods Hole. It might be said that all this only proves that the activating effect requires a smaller concentration than the agglutinating effect, but may yet be caused by the same substance. This objection is, however, not tenable in the following case.

Purpuratus sperm washed in *m*/2 NaCl is as a rule more active in a mixture of NaCl + KCl than in a mixture of NaCl + $CaCl_2$ (if both solutions are free from egg contents); yet in the latter solution the agglutination reaction upon the addition of egg-NaCl is very strong while in the former it is lacking (unless the sperm or testicles or ovaries give off some $CaCl_2$ to the surrounding solution). Again it might be argued that the activation of the spermatozoon might be induced by the same substance as the agglutination, but that the agglutinating substance in both cases reacted with different constituents of the spermatozoon. While this may be admitted, it must also be conceded that with the facts which we have at our disposal at present we can not be certain that the aggultinating and activating substances are identical.

VII

Lillie[22] not only takes the identity of the two substances for granted but he assumes that without the agglutinating substance in the egg (to which he gives the somewhat prejudicial name "fertilizin") no fertilization is possible. Fertilization in his opinion consists in the combination of the spermatozoon with a molecule of "fertilizin" in

[22] *Loc. cit.*

the egg, whereby the fertilizin molecule undergoes a change in the other end and this change causes the egg to develop. The fertilizin is thus an "amboceptor" in the sense of Ehrlich's side-chain theory.

The side-chain theory was invented by Ehrlich for an altogether different purpose. Bordet had found that for certain phenomena of immunity two substances were needed (which Ehrlich named amboceptor and complement, respectively). Ehrlich assumed that they were bound chemically by the antigen (the substance against which the organism was immunized) but found that while the antigen (*A*) was able to bind *B* (the amboceptor) in the absence of *C*, it was not able to bind the complement *C* in the absence of *B*. From this Ehrlich concluded that of the two possible modes of linkage between the three bodies $A\genfrac{}{}{0pt}{}{\diagup B}{\diagdown C}$ and $A-B-C$ the latter was the one which really occurred. Since in this case *C* is not directly linked with *A* but through the intermediation of *B* he called *B* the "amboceptor" and the scheme of linkage a "side-chain" linkage.

Lillie applies this theory (which covers the two possible modes of linkage of two chemical compounds to a third one) to the entrance of the spermatozoon into the egg, by calling the egg an antigen *A* and the spermatozoon a complement *C* and assuming the existence of a hypothetical amboceptor *B* in the form of the substance that causes agglutination, the "fertilizin." Even if we are willing to overlook the fact that the egg and the spermatozoon are cells and not simple organic compounds and if we are willing to overlook the further fact that the assumption of an amboceptor as a connecting link between the two is arbitrary we can not overlook the fact that the spermatozoon does not combine chemically with the egg but that it actually enters into the egg and attaches itself to the egg nucleus. It seems then futile to discuss whether the spermatozoon combines with the egg in side-chain fashion (namely, Egg—Fertilizin—Spermatozoon) or in direct

fashion, namely,

Egg ⟨ Fertilizin
Egg ⟨ Spermatozoon

since the engulfing of the spermatozoon into the egg is a physical process which bears no relation to either possibility.

It has been stated that the "fertilizin theory" explains also the phenomena of artificial parthenogenesis just as well as any other theory. In a recent book on artificial parthenogenesis the writer has given the results of a large number of experiments and he has tried to explain some of them; the reader would, however, vainly look for a "theory" of artificial parthenogenesis. A theory in a scientific sense consists in the presentation in mathematical or numerical form of a phenomenon as the function of its variables. The writer has tried to prepare the ground for such a treatment of the phenomena of fertilization and of the first development of the egg by working out those variables which permit a quantitative treatment, but even if the exploration had been advanced further than it actually has been, it would not be possible to ever expect that a single theory could cover all the phenomena of fertilization and development, since under these two headings so many physically and chemically different processes are included (of which one follows the other) that they can not be covered by one theory. It is true the writer had in former publications occasionally used the term "lysin theory of fertilization" but only to express the fact that cytolytic agencies induce membrane formation and that the membrane formation induced by a spermatozoon might also be due to a cytolytic agency contained in the spermatozoon; but he has dropped this term in his recent book on the subject.

While the writer does not desire to enter into a further discussion of the side-chain theory of fertilization he wishes to point out that it rests on the claim that that substance which causes sperm agglutination is contained

in the unfertilized egg and that the egg can only be fertilized as long as this "fertilizin" is present in the egg. It is obvious that such an assumption demands for its proof that in all cases in which an egg can be fertilized it must contain the agglutinating substance. There is only one test for the presence of this substance, namely the cluster formation of the sperm in the presence of egg sea water. This proof can not be furnished since, as the writer had shown in a former paper, the reaction is lacking in many cases of hybridization; it is also lacking in the case of the starfish.[23] It is not impossible that if the theory is tested further it will be found lacking in a considerable number of cases. To this objection Lillie replies that it is not necessary that the eggs should actually give the agglutinin reaction, it is sufficient that the agglutinating substance is contained in the egg. But how can we tell that it is contained in an egg which fails to give the agglutination reaction as long as this reaction is the only reliable test for the presence of the agglutinating substance in the egg? Rigorously speaking, even if all eggs of every species gave the agglutinin reaction it would still be necessary to furnish a direct proof that the agglutinin has anything to do with fertilization and development.

It may be possible that Lillie considers such a proof to be contained in the following statement.

> I adopted then the working hypothesis that this substance[24] is necessary for fertilization and there followed immediately three corollaries, viz.: (1) if it were possible to extract this substance from eggs they would no longer be capable of fertilization; (2) fertilized eggs are incapable of uniting again with spermatozoa, hence if the hypothesis is correct they could no longer contain free fertilizin; (3) eggs in which membranes have been formed by methods of artificial parthenogenesis become incapable of fertilization; such eggs must also therefore be devoid of free fertilizin after they have reached the non-fertilizable condition if the hypothesis is correct. These consequences were actually found to be true.[25]

[23] Lillie, *Biol. Bull.*, XXVIII, 18, 1915.

[24] The "fertilizin."

[25] Lillie, *Jour. of Exper. Zool.*, XVI, 523, 1914.

Of these three "corollaries" the first one is the most important, since it claims that the power of the eggs of being fertilized varies with their contents of fertilizin. The proof consisted in this: that eggs were washed a number of times during three consecutive days and after two days the percentage of eggs that could be fertilized were diminished to about one third.

> There is thus the anticipated decrease in the percentage of fertilizations.

It is a well known fact that the unfertilized eggs of the sea urchin (in fact of all marine animals) perish when they lie for some time in sea water and one of the main causes of this phenomenon is also known, namely oxidations. If the oxidations are inhibited through the removal of oxygen or the addition of KCN the life of the eggs can be prolonged.[26] In the mature starfish egg this death which is accelerated by the temperature (and has the high temperature coefficient of many life phenomena) takes place in a few hours,[27] while it begins a little later in the egg of the sea urchin. After the artificial membrane formation it takes place very rapidly also in the sea urchin egg (coincident with the enormous increase in the rate of oxidations caused by the artificial membrane formation) and in this case the death of the egg can also be retarded by the withdrawal of oxygen or the addition of cyanide.[28] In view of these facts the objection can not be avoided that in Lillie's experiment the number of eggs which could be fertilized fell off after two days to one third not on account of the loss of "fertilizin" but because of the fact that two thirds of the eggs were dead by that time. That this assumption is well grounded is testified by Lillie's own remarks:

> Concomitantly, with these effects of the series of washings the developmental energy becomes greatly reduced. This was very obvious from the second fertilization.[29] On August 24 (48 hours after fertilization) a large quantity of living material was contained in the second A fertili-

[26] Loeb and Lewis, *Am. Jour. Physiol.*, VI, 305, 1902.

[27] Loeb, *Biol. Bull.*, III, 295, 1902.

[28] Loeb, "Artificial Parthenogenesis and Fertilization."

[29] Which occurred on the second day.

zation but none had even approximately pluteus structure. The most common form was a stereoblastula. In the second *B* fertilization there were a few abnormal prismatic plutei, while the majority were gastrulae. The third fertilization resulted in extremely abnormal ciliated types. The fourth and fifth did not proceed beyond abnormal cleavage stages. From this and similar experiments Lillie draws the following conclusion:

> The eggs have evidently lost something which affects their power of fertilization. Table 3 shows the measure of loss of the sperm agglutinating substance and justifies the general conclusion that this is a factor in the result. The loss of other substances may also combine in the decrease of fertilizing power, but of this we know nothing definite. As a matter of fact, fertilizing power is gradually lost with decrease of fertilizin content of the egg.

It seems to the writer that in these experiments the power of being fertilized was gradually lost by the death of the eggs. And an additional justification of this criticism is given by the following fact, that if we deprive *fresh* eggs of *purpuratus* permanently of their power of giving off "fertilizin" their power of being fertilized is not only not lost but is entirely unaltered. The writer has shown that if the eggs of *purpuratus* are treated for two or three minutes with a mixture of 50 c.c. of sea water + 3 c.c. of HCl (whereby the jelly surrounding the egg is dissolved) and if the eggs are washed they give no trace of a fertilizin reaction but 100 per cent. of the eggs can be fertilized.[30]

It might be argued that the supernatant sea water from these eggs had not lost all power of causing agglutination of the sperm. This the writer must deny but for arguments' sake he will admit that a trace near the "psychological limit" might have been overlooked where a "fertilizin" partisan might have declared that he still could perceive a faint indication of a "fertilizin" reaction. In that case only a few eggs should have been fertilized—the fertilizin theory rests on this assumption; in reality, however, practically one hundred per cent. were fertilized in every case (provided the eggs had not been lying in the see water too long, *i. e.*, more than a day or two).

[30] Loeb, *Jour. Exper. Zool.*, XVII, 123, 1914.

To this Lillie replies that perhaps the sperm of *purpuratus* is not so delicate an indicator for agglutinin as the sperm of *Arbacia*—but as long as the agglutination reaction is the only test for the presence of fertilizin in the egg, such an answer begs the question.

From the fact that the power of agglutinating the sperm is lost if the egg of *purpuratus* is deprived of its jelly by acid treatment the writer drew the conclusion that in this egg the "fertilizin" does not come from the unfertilized egg but only from its jelly and that this was contrary to Lillie's assumption. To this Lillie[31] replied by pointing out that the immature eggs of *Arbacia* do not give the agglutination reaction while the mature *Arbacia* egg gives the reaction very powerfully, and that we must conclude from this that the "fertilizin" contained in the jelly comes from the egg and is given off during the period of the maturation divisions (the latter statement, however, is after all only an assumption though a probable one). But this does not meet the question at issue, namely that in the egg of *purpuratus* at the time of maturity the fertilizin which is given off is contained exclusively in the jelly and not in the egg, as it should be if the presence of fertilizin in the egg were a prerequisite for its ability of being fertilized. It is true that if we repeat this experiment in the egg of *Arbacia* we find that after the removal of the jelly by HCl a trace of the agglutinating substance may still be given off by the egg, although little in comparison with that given off by the jelly. But this does not alter the facts as they are found in the egg of *purpuratus*.

As far as the two other proofs of Lillie are concerned, we have already touched upon them in the previous parts of this paper. The fact that the fertilized eggs of *Arbacia* (and of *purpuratus*) cease to give the agglutinin reaction is due to the loss of the jelly on the part of the fertilized egg to which in *Arbacia* should be added the fact that some of the material of the cortical layer is given

[31] Lillie, *Biol. Bull.*, XXVIII, 18, 1915.

off during the process of membrane formation. The writer has pointed out in former papers that the cortical layer of the egg which undergoes liquefaction in the process of membrane formation behaves towards reagents very much like the jelly which surrounds the egg.[32] But since in the egg of *purpuratus* the loss of this agglutinating power on the part of the egg is not necessarily accompanied by the loss of the power of being fertilized—*e. g.*, in the HCl experiment—we are inclined to believe that there must be another reason that an egg fertilized by sperm can not be fertilized a second time.

As far as the statement is concerned that the egg can no longer be fertilized after artificial membrane formation by butyric acid the writer can not admit the correctness of this statement (see Chapter III). In the eggs in which artificial membrane formation has been called forth by butyric acid the main if not the only block to a subsequent fertilization is the membrane itself.

This can be proved by a very simple experiment. If we call forth the membrane formation in the egg of *purpuratus* in a neutral or faintly alkaline solution of $m/2$ ($NaCl + KCl + CaCl_2$) (instead of in sea water) a very thin membrane is formed, which is easily torn and offers no resistance to the spermatozoon. All the eggs treated in this way can be fertilized by sperm. The agglutinin reaction of such eggs is, however, permanently lost.

The facts thus far known seem to force us to the conclusion that no adequate proof has been offered thus far for the connection between the power of an egg of being fertilized by sperm and its power of causing a cluster formation of the sperm. The writer has pointed out in a previous paper that it is difficult to see why there should exist such a relation, since sperm agglutination can only inhibit the entrance of the spermatozoon into the egg.

[32] "Artificial Parthenogenesis and Fertilization," Chicago, 1913, pp. 210–14. University of California publication, Physiology, Vol. 3, p. 1, 1905.

GERM CELLS AND SOMATIC CELLS[1]

LEO LOEB

Results obtained in the field of experimental pathology and especially in cancer research have an important bearing on certain problems of general biology. In the following I wish to consider connectedly some of these facts from this point of view.

I. A sharp distinction between germ cells and somatic cells has become clearly established, especially through the writings of Nussbaum and Weismann. More recent results which demonstrated that the differentiation of germ cells from the somatic cells at a very early stage of embryonic development and their non-participation in the formation of somatic tissues exists in various species, tended to emphasize this sharp distinction between somatic and germ cells.

Weismann[2] especially insisted on the radical difference between germ cells and somatic cells, inasmuch as he attributed potential immortality to the former and only a temporary existence to the latter. And Weismann regards this difference as essentially founded in the structure of both kinds of cells and fundamentally connected with the functioning of the somatic cells; this difference was obtained through selective processes as an adaptation in the struggle for existence. He does not regard the death of somatic cells as an accidental occurrence due to unfavorable conditions which it might be in our power to change, but as an inherent characteristic of somatic cells. He mentions, though casually, that the life of the cock's comb might be prolonged by grafting it on another fowl—but only to dismiss this idea as having no important theo-

[1] From the Department of Pathology, Barnard Free Skin and Cancer Hospital, St. Louis.

[2] A. Weismann, "Ueber Leben und Tod," Jena, 1884; "Ueber die Vererbung," Jena, 1883.

retical bearing. R. Hertwig[3] also regards the death of the somatic cells as unavoidably determined by their organization which precludes necessary readjustments.

Minot[4] likewise held the life of somatic cells to be limited in duration and he ascribed this limitation to changes in cell structure, leading to a differentiation of the cytoplasm during the process of life; a change which he designated as cytomorphosis.

Within the last 14 years certain facts have been established which are contrary to this conception of a radical difference between germ and somatic cells as far as their potential immortality is concerned. Experimental investigations in tumor growth have furnished these facts. Before we state these results we have first to consider, how far tumor cells can be regarded as somatic cells. We consider here malignant tumors (cancers). They originate at various parts of the body, often under the influence of long-continued irritation. In many cases we can, if we obtain sufficiently early tumors, trace the transformation of the normal into the abnormally proliferating (tumor) tissue. This has been, as was to be expected, especially observed in the case of superficial cancers, where early stages of tumors are most likely to be encountered, for instance, in cancers of the skin, of certain mucous membranes, but also occasionally in internal cancers as in those of the stomach. In such cases the cancer cells are undoubtedly the offspring of ordinary somatic cells. There are, however, tumors, so-called teratomata, which in all probability take their origin in the germ glands and other parts of the body from parthenogenetically developing ova. But while these latter tumors do not originate from somatic, but from germ cells, the tumor cells themselves are no longer germ cells, but somatic cells in the same sense as the ordinary tissue constituents which also are derived from germ cells. We can therefore

[3] R. Hertwig, *Biol. Centralblatt,* Bd. XXXIV, No. 9, 1914.

[4] C. S. Minot, "The Problem of Age, Growth and Death," New York, 1908.

without doubt regard tumor cells as a kind of somatic cells.

One of the most characteristic properties of cancer cells is their ability to grow after transplantation into other animals of the same species. This applies not to all, but to a certain number of spontaneous cancers; the majority of spontaneous tumors are not transplantable into other individuals of the same species. They grow, however, usually after transplantation into the same individual in which they originated. There does not exist as far as their origin is concerned any essential difference between these two kinds of cancers—those transplantable and not transplantable into other individuals. The cancers used in experimental tumor investigation take their origin from somatic cells; but it appears some are less sensitive to the difference in the chemical composition of the body fluids which exists between different individuals of the same species than others, and those less sensitive can be transplanted, while others can not.

In those tumors which are transplantable, relatively few tumor cells give after inoculation into other animals origin to the new tumors, and the tumor cells after the first transplantation not rarely multiply with greater vigor than they did in the original animal, an effect caused, as I could show, through the stimulating influence of the cutting and otherwise manipulating the tumor cells. In each animal therefore there are produced many successive generations of tumor cells, and after transplantation into another individual each surviving cancer cell produces again new generations. Consecutive transplantations into many individuals have been carried out with the same tumor. The potential proliferative power of the cancer cells is therefore enormous. It is, however, not so much the intensity of the proliferative power of the tumor cells which we wish to consider as the potential duration of their life. It has been shown that epithelial, as well as connective tissue tumors can be transplanted through many generations and can survive for a long time the animal in which the tumor originated. Thus I was able

to transplant the connective tissue cells of a rat sarcoma through forty successive generations of animals, and it was merely the result of accidental bacterial infection due to the unfavorable conditions under which the work had to be carried out which caused the ultimate death of the propagated cells.

An epithelial tumor found by Jensen in a mouse has been propagated in various laboratories through a period of almost fifteen years, and another epithelial tumor of the mouse we have been propagating in mice for a period of seven or eight years, without any sign of diminishing vitality in the propagated cells being noticeable.

In all these transplantations of tumor cells, be they of connective tissue or epithelial origin, it could be shown that the peripheral cells remain alive and from these surviving cells the cell growth starts. These observations suggested to me in 1901 the conclusion that tumor cells may have a potential immortality in a similar manner as germ cells,[5] and inasmuch as tumor cells are only modified somatic cells, I furthermore concluded that the same statement holds good in the case of somatic cells.[5] Further experiences in the field of experimental tumor investigation during the following years confirmed this conclusion and permitted its enunciation with greater definitiveness.[6] The potential immortality of the somatic cells of course can only be made probable, it can never be definitely proved, inasmuch as our experience merely deals with finite periods. But the same restriction holds good in the case of the germ cells in which the potential immortality is likewise merely a strong probability and not a definitely proven fact.

Weismann believed that protozoa are in the same sense potentially immortal as germ cells, in contradistinction to somatic cells which do not possess potential immortality. Some facts were, however, discovered which, according to

[5] "On the Transplantation of Tumors," *Jour. Medical Research*, Vol. VI, No. 1, 1901, p. 28; *Virchow's Archiv*, Bd. 167, 1902, p. 175.

[6] "Tumor Growth and Tissue Growth," *Am. Philosophical Society*, XLVII, 1908, and at other places.

the interpretation given them, seemed to contradict Weismann's conception. Thus Maupas found that various kinds of infusoria did not propagate by fission indefinitely, but that a sexual process, conjugation, was necessary at certain times, and Calkins showed that there were regular periods of depression, and while a spontaneous recuperation from the effects of certain depressions could take place and in still other cases artificial stimulation would aid the animals in overcoming the critical periods, at other times depressions proved fatal without an intervening conjugation. Woodruff, however, by choosing conditions of environment more in accordance with the conditions found in nature, could keep a strain of *Paramœcium* apparently indefinitely alive without any intervening periods of copulation being required. This seemed to point to a potential immortality of protozoa in the sense of Weismann. Recently, however, Woodruff and Erdmann[7] found that the recovery from depression which takes place is accomplished through nuclear changes comparable to, but not identical with, those observed during copulation. This seems in some respects to agree with R. Hertwig's previously enunciated theory according to which depressions and senility in cells are due to a disproportion between the nuclear and cytoplasmic material, and that recovery from such unfavorable conditions depends upon the reorganization of the nucleus, essentially consisting in a diminution of the mass of the latter. Inasmuch as in metazoa—he concluded, further—such a rearrangement between nuclear and cytoplasmic masses can only take place in the case of germ cells, but not somatic cells, only germ cells are immortal, while somatic cells are necessarily mortal. While Weismann regarded the unavoidable mortality of the somatic cells as a secondary acquisition, the result of a process of selection, the death of somatic cells being of advantage to the propagation of the race, Richard Hertwig[8] regards the death of somatic cells as inherent in their structure, which precludes the

[7] *Biol. Centralblatt*, Bd. 34, August, 1914, p. 484.
[8] *Biol. Centralblatt*, Bd. XXXIV, 1914, No. 9.

possibility of nuclear reorganization of the cell necessary for continued life. In a somewhat related way, Minot considers, as mentioned above, the death of somatic cells as inevitable and as the result of cytomorphosis, which means the relative increase in size and differentiation of somatic cells during life. In this connection it is interesting to note that while R. Hertwig considers (in protozoa primarily, but secondarily also in other cells) an increase in the size of the nucleus—the result of the activity of the cells—as the cause of functional disturbances leading to senility, Minot on the other hand connects senility with a relative decrease in the size of the nucleus and an increase in the mass of the cytoplasm. Now as far as the protozoa are concerned, the controversy does not seem to concern so much the potential immortality of these organisms as the problem as to whether the individual protozoon corresponds to a germ cell or to a somatic cell of a metazoon, or whether it partakes of the character of both. There can be little doubt that individual protozoa possess potential immortality, a conclusion which would not be invalidated through a loss of certain parts of the protozoon body at certain periods of its life cycle.

We may therefore conclude that all three kinds of cells, protozoa, germ cells, as well as certain somatic cells of metazoa, possess a potential immortality.

Tumor cells are somatic cells in which such secondary changes leading to a cessation of proliferation as take place under certain conditions in all the individual cells in some kinds of somatic tissues, are affecting only a certain number of cells. In the case of some somatic cells, as, for instance, those of the epidermis, it is evident that the secondary changes in structure and metabolism, which lead to a cessation of proliferative power, are due to unfavorable conditions of blood-supply. What Minot calls cytomorphosis can therefore in this case be referred not to necessary transformations inherent in the cells, but to unfavorable environmental conditions into which the cells are placed as a result of their multiplication. Such secondary degenerative changes take place also in tumor

cells under similar defective conditions of blood-supply. Here also degenerative changes entail a cessation of proliferation in a similar manner as in ordinary tissue cells. While farther away from the blood-vessels the tumor cells degenerate and die, near the blood-vessels they continue to live and to multiply.

While from a theoretical point of view, therefore, the question as to the potential immortality of somatic cells has through the experiments on tumor cells been answered in a decisive manner, it was nevertheless of interest to extend these investigations to ordinary tissues. Such investigations we undertook in the course of the last eight years, and while certain obstacles were encountered, which prevented the continued life of ordinary tissues, the results were of interest in giving an insight into some of the conditions which determine the growth, life and death of somatic cells. These investigations have shown that if tissues are transplanted into another individual of the same species, under the influence of the constitution of the body fluids, which differs in different individuals of the same species, the metabolism in the transplanted tissues is interfered with as shown, for instance, in pathological differences in pigmentation seen in black skin of the guinea-pig after transplantation into other animals of the same species. After transplantation of pigmented skin into the same individual in which it originated, such pathological changes do not occur. As I have previously pointed out, a certain adaptation exists between the tissues and body fluids in animals of the same species, and even between the tissues and body fluids of the same individual. Thus it comes about that the interaction of tissues and body fluids of the same species leads to different and less toxic products than those produced through the interaction of the body fluids of one with the tissues of another species. Even the interaction of tissues of one animal with the body fluids of another animal of the same species leads to more toxic products than the interaction of body fluids and tissues of the same individual. In the latter case toxic products, interfering with the life of

normal tissues, are not produced, while in the former cases such products acting directly or indirectly are formed. As an illustration of such a specific relationship between body fluids and tissues, I cited the specifically adapted effect which tissue coagulins exert on the constituents of blood plasma.[9]

Now as the result of these differences in metabolism induced through the differently constituted body fluids the lymphocytes begin to invade the transplanted tissues, and the invading connective tissue does not preserve, as it does after auto-transplantation, its young and cellular state, but produces fibrous bands which contract around the parenchyma after homoiotransplantation, and thus exert pressure. Both connective tissue and lymphocytes destroy thus the homoiotransplanted tissue, while they usually spare the autotransplanted tissue the metabolism of which is normal. In the case of certain tissues, as, for instance, kidney, however, even after autotransplantation into the subcutaneous tissue the metabolism of the transplanted cells becomes abnormal under the abnormal conditions under which they now live, and here the lymphocytes and connective tissue destroy, therefore, even the autotransplanted tissue, although at a later date than the homoiotransplanted kidney tissue.

The fitness of a tissue in an individual determining its power to live or to grow depends, therefore, on two factors: (1) on the specific adaptation existing between tissues and body fluids, and (2) on the way in which various substances are carried to the tissue. A perfect nutrition implies the carrying of the food substances to the tissues in the normal way through blood-vessels. It is probable that on the intact relations between capillary endothelium and parenchyma cells depends such a sifting of various food substances and waste products as is best suited to the normal metabolism of the cells. If, as in the case of the kidney tissue, this mechanism is disturbed, abnormal substances are produced notwithstanding the specific adaptation existing in this case between tissue cells and body

[9] "Immunity and Adaptation," *Biol. Bulletin*, Vol. IX, 1905, p. 141.

fluids after auto-transplantation. And it seems that a perfect fulfilment of the second requirement might even be able to overcome a deficiency in the first condition, the specific adaptation between tissues and body fluids. This seems at least to be the case, whenever a kidney is successfully transplanted into another individual of the same species and lives here for a long period of time.

A peculiar resistance to foreign body fluids is apparently shown by the germ cells. They represent in reality individuals residing in a host organism of the same species. In this case the host organism is nearly related to but not identical with the individuality of the germ cells. In some respects we have, therefore, here a condition comparable to one existing after homoiotransplantation of tissues. And still the germ cells do not show any signs of injury. There is, therefore, in germ cells as yet lacking that substance which has a specific affinity to certain parts of the body fluids, or through their situation the germ cells are somehow protected against the injurious influence of these substances.

Thus it comes about that through transplantation into other individuals of the same species the potential immortality of the ordinary tissues can not be demonstrated. This applies to the tissues investigated so far.[10] However, it is quite possible that we may yet find that in the case of certain tissues the life may be permanent even after homoiotransplantation. It was furthermore thinkable that through serial transplantation, retransplanting the tissue at an early date before the lymphocytes and connective tissue had had a chance to seriously injure it, better success could be obtained. In the case of the skin I have undertaken such serial transplantations some years ago; our investigations have, however, in this case shown that it was not possible to retransplant this particular

[10] Leo Loeb u. W. A. F. Addison, *Arch. f. Entwicklungsmechanik*, Bd. XXVII, 1909, p. 73; Bd. XXXII, 1911, p. 44. Max W. Myer, Bd. XXXVIII, p. 1, 1913. Llewellyn Sale, Bd. XXXVII, 1913, p. 248. M. G. Seelig, Bd. XXXVII, 1913, p. 259. Cora Hesselberg, *Journ. Experimental Medicine*, Vol. XXI, 1915, p. 164.

tissue indefinitely;[11] these experiments ought to be extended; especially might it be of interest to use the direct descendants as hosts for the tissues of the parent. It is to be expected that the quality of the parents which makes the body fluids suitable for their own tissues might make them likewise suitable for certain of their offspring. Such experiments I began some time ago and I expect to continue them if opportunity should present itself.

The growing of tissues in culture media, which excludes attack on the cells by connective tissue and lymphocytes, may also serve the same purpose and quite recently has been used through a larger number of generations. But, as stated above, we have in these experiments merely to deal with an attempt to confirm the potential immortality of the somatic cells which had in principle been established through previous investigations on the life of tumor cells.

While thus various kinds of tissues of an organism have the potentiality of an immortal life, separated from the organisms to which they belonged, the organism as a whole invariably dies and with it its component tissues. This is evidently due to the interdependence of various parts of an organism and to the death of certain sensitive cells, especially the ganglia cells of the central nervous system. We might therefore be inclined to conclude that these ganglia cells do not possess the potentiality of immortal life. But even in the case of the ganglia cells, which are of such significance for the life of the organism as a whole, we can at present not deny the possibility that they also may have the potentiality of immortality and that they merely succumb under the influences of certain injurious conditions arising in the organism. On the other hand, fully developed ganglia cells have apparently lost the power to multiply; they are furthermore sensitive to certain insults to which other tissues show resistance. Thus the unfavorable condition prevailing during the process of transplantation into another organism and directly afterwards seems to be sufficient to cause the death

[11] Leo Loeb, *Archiv. f. Entwicklgsmch.*, Bd. XXIV, 1907, p. 638.

of certain ganglia cells when other tissues would survive. But neither of these facts prove that under favorable conditions very much differentiated cells, like ganglia cells, might not have the power to live indefinitely, although they have lost the power to multiply. Sensitized connective tissue cells of the uterus may after transplantation into the same or another individual in which corpus luteum substance is circulating grow very energetically and produce placentomata, while after transplantation of fully developed deciducomata no further growth can be obtained and all or almost all the cells die. Here also the fully "differentiated" cells have lost the power to multiply and at the same time they have apparently become more sensitive to the effect of injurious influences than young and yet undifferentiated predecidual cells of the uterine mucosa.

But here we can observe that some strands of fully differentiated placentoma tissue may survive even under those unfavorable conditions—without, however, resuming growth or returning to the undifferentiated condition—namely, such strands of tissue as are situated under the best environmental conditions, in close proximity to the host tissue, at places most accessible to the foodstuffs or oxygen supplied by the circulating blood or by the peritoneal fluid. This suggests that even much differentiated cells which have lost their power to propagate may still have the power to live, when kept under favorable conditions, and that their death is the result of unfavorable environmental influences. Thus we must at least admit at the present time the possibility that also the ganglia cells, while they do no longer multiply, may still possess a potential immortality; that cellular differentiation precludes the latter possibility has as yet not been demonstrated. We must therefore sharply distinguish between the power of cells to grow and their power to live; while the former seems to be destroyed through differentiation—at least in some cases—the latter may still exist. In the case of other tissue cells we have it to a certain extent in our power through experimental conditions

to prevent those changes which lead to differentiation and death; thus in the case of tumor cells through constant transfer into a new host we can enormously increase the number of as yet less differentiated cells on which the propagation depends by causing an intense multiplication of these tumor cells, many of which, if left in the same organism, would have undergone secondary degenerative changes. Through experimental means, viz., through the transplantation into different kinds of hosts the relative preponderance of propagation and differentiation of tumor cells can be varied. We furthermore know that through "chemical" sensitization combined with mechanical stimulation, the same effect can be produced, at least temporarily, in the connective tissue cells of the uterine mucosa. Under those conditions a large number of cells are induced to propagate and remain young while their offspring gradually change into fully differentiated cells. If we grant the possibility that differentiation of such cells as ganglia cells, while it entails loss of the power to propagate and greater sensitiveness to insults, does not necessarily mean the necessity to die, then the problem of prolongation of life would to a great extent depend upon the possibility of preventing injurious influences which at present disturb the function of ganglia cells from attacking these cells and causing their death.

II. The germ cells are potentially immortal, but this potential immortality can only be realized, if at certain periods certain changes take place within the cells, which concern especially the nucleus, phenomena consisting in maturation, followed by fertilization or parthenogenetic development. In a similar manner the observations of Woodruff and Erdmann suggest that at the time of depressions in the life of the protozoa, possibly similar nuclear phenomena take place at least in certain cases. We have seen that in the case of somatic cells there are also indications of the existence of potential immortality. The question may therefore be raised, whether similar periodic rearrangements of the nucleus, as in the case of the germ

cells and protozoa, may not also take place in the case of the somatic, especially of tumor cells. Without considering any connection with the problem of the immortality of the somatic cells, Bashford, Murray and Bowen[12] stated on an empirical basis that in charting the number of successful inoculations of a mouse carcinoma in mice, in different generations and in different strains of the same generation, they noticed definite rhythmic variations in the number of successful inoculations, a maximum of successful inoculations in one generation being followed by a minimum in the succeeding generation. However, they also state that parallel strains of the same tumor did not show maxima or minima at the same time. Bashford, Murray and Bowen, in order to explain these observations, assumed that different parts of the same tumor show different degrees of growth energy at the same time; this would imply that such areas differing in growth energy at the same period are separated through transplantation; so that in one generation mainly energetically growing pieces are used for transplantation in the succeeding weakly growing pieces almost altogether, an assumption which does not appear very probable.

Calkins[13] held that there occur in succeeding generations not so much rhythmic variations in the number of successful transplantations as in the growth energy of the tumors. He compared these rhythmic variations with the rhythms observed by him in the case of *Paramæcium.* However, such rhythms were not noticeable in the mouse carcinoma which we have propagated for a number of years in our laboratory, as has been shown by Moyer S. Fleisher.[14] He furthermore shows that even in the case of those tumors, on the study of which Bashford and his collaborators and Calkins base their conclusion, it is very probable that the variations which these authors observed do not represent definite rhythms, but are, as far

[12] Bashford, Murray and Bowen, *Zeitsch. f. Krebsforschung*, 1907, Bd. 5, Heft 3.

[13] Calkins, *Jour. Exper. Med.*, 1908, X, 283.

[14] Moyer S. Fleischer, *Zeitschrift f. Krebsforschung*, Bd. 14, Heft 1, 1914.

as their conclusions are not based on methods of determining the growth energy of tumors, not suitable for this purpose, in all probability merely the expression of the existence of a number of uncontrolled variable factors. Such factors are numerous and they may explain certain variations observed in growth energy and number of takes in transplantations undertaken at different times or in different mice. There exists, therefore, at the present time no evidence making even probable the existence of rhythms of growth and vitality in somatic cells comparable to those found in protozoa; neither have thus far been found in somatic cells indications of nuclear changes similar to those periodically occurring in germ cells and probably also in some protozoa and apparently bearing some relation to variations in growth and vitality in these cells. At present we must therefore reckon at least with the possibility that the immortality in somatic cells is not connected with rhythms in vitality and in nuclear changes of such a character as observed in the other two kinds of potentially immortal cells.

III. External factors acting on an organism may exert an influence on its germ cells and here produce certain changes which may be transmitted to the following generations, and thus through a number of generations the offspring may show deviations from the type, although the character of the lesions appearing in different generations may not be identical. This has been observed as a result of the action of poisons such as lead and alcohol. Especially the extensive investigations of Stockard on the action of alcohol in guinea pigs demonstrate conclusively that defects appear through several generations. In the case of these injuries transferred to the offspring, it is doubtful whether and to what extent these inheritable defects are characteristic for a certain poison, or whether we have to deal with traumatisms which might be caused in a similar manner by many poisons or even by injurious physical agencies. There is some evidence tending to show that most diverse chemicals may influence embryonic development in a similar manner, that they may produce

identical defects in the developing organisms. It seems to be otherwise in the case of certain external conditions which produce first changes in some somatic cells which on their part apparently induce such secondary changes in the germ cells that in the offspring, not exposed to the external conditions that affected the parents, again changes appear in some somatic cells similar to those produced in the parents through the external conditions. Such results were published by Kammerer. In the latter case the changes produced and transmitted to the offspring showed evidently a characteristic specific relationship to definite external conditions. Such a specific relationship is, as far as we can judge at present, lacking in the case of defects or deficiencies produced through the action of poisons.

I wish to report briefly on a change which my collaborators Moyer S. Fleisher, Miguel Vera and myself[15] have produced in somatic cells, a change which is transferab'e to the following cell generations and is therefore hereditary, and which, while it would be pronounced non-specific, if we should use ordinary criteria, can through the use of special methods be shown to possess a definite, characteristic relationship to the external factor that caused this change and must therefore be called specific. The observations on which these conclusions are based are briefly as follows:

If we inoculate mice with the mouse carcinoma used by us in our experiments and from the ninth to the fourteenth day after inoculation give on successive days four intravenous injections of such substances as colloidal copper or hirudin, a marked inhibition in growth takes place during the period of injection. The intensity of this inhibition varies in different cases and it is possible for us by using a combination of two substances to cause a retrogression of a considerable number of tumors. Now,

[15] Moyer S. Fleisher and Leo Loeb, *Jour. Exper. Med.*, Vol. XX, 1914, p. 503. Moyer S. Fleisher, Miguel Vera and Leo Loeb, *Jour. Exper. Med.*, Vol. XX, 1914, p. 522. Moyer S. Fleisher and Leo Loeb, *Jour. Exper. Med.*, Vol. XXI, 1915, p. 155.

if we inject daily from the second to the fifth day after inoculation mice with either of these two substances, tumor growth is not noticeably retarded through the injections. Tumors, in an early stage of development, are resistant to this inhibiting effect. If we now give four intravenous injections, one on each successive day, from the second to the fifth day, and later again to the same mice four injections from the ninth to the thirteenth day, the latter series of injections which had been effective in other animals not previously injected from the second to the fifth day, have now almost completely lost their efficacy. The mice have through the first set of injections become immune against the action of colloidal copper and hirudin as far as the effect of these substances on tumor growth is concerned.

We next inquired into the mechanism of this immunity, and especially were we concerned with the place where this immunity is produced. It was conceivable that this took place either in some organ of the injected animal or in the tumor cells themselves. The following experiments showed that both possibilities were realized: If we inject the mice on the four days preceding inoculation with tumor, immunity is produced, at least in the case of colloidal copper. This proves that some organ in the host animal contributes to the immunity, inasmuch as in this case the preliminary injections exerted their influence without having had a chance to act on the tumor cells. But the tumor cells themselves also become actively immune, as shown in the following manner: We inject animals with either colloidal copper or hirudin from the second to fifth day, and again from the ninth to the thirteenth day after inoculation. Two days after the last injection we used the tumors of some of the injected animals for reinoculation into a new set of mice. Nine to thirteen days after this second inoculation the mice belonging to the second set are injected with colloidal copper and hirudin, respectively. Now we find that these tumors also are almost entirely resistant against the effect of colloidal copper and hirudin, although in this case no preliminary injections had been given to the animals which

are now the bearers of the tumors. It is therefore necessary to conclude that the tumor cells used for transplantation are in this case the sole bearers of the immunity, and that the tumor cells themselves have been actively immunized. From the latter experiments we may furthermore conclude that the immunity acquired by tumor cells is transferred to the following generations of tumor cells, and that therefore a hereditary transmission of a character acquired by somatic cells under the influence of external conditions takes place. The conclusion is based on the following consideration: The process of tumor inoculation consists in the transfer of a very small particle of tumor. Very soon after transplantation most of the transplanted tumor cells become necrotic and only a relatively small number of peripheral tumor cells remain alive. These very soon begin to proliferate, and through their proliferation give origin to the developing tumor. If therefore the tumors developing after transplantation in the new hosts are immune, the immunity must have been transmitted from the few cells remaining alive after inoculation to the new cell generations to whom they give origin. A fully developed tumor represents a combination of a large number of generations of tumor cells and it may be assumed that the later generations of tumor cells preponderate numerically very much over the earlier generations. Through how many generations of tumor cells this transmission of the acquired immunity can be propagated remains yet to be determined. From our preliminary experiments, which are, however, not yet definite, it appears not improbable that it extends at least through several series of transplantations.

Both colloidal copper and hirudin inhibit tumor growth. The sign by which we judge the effect of these substances is therefore essentially the same in both. We might thus be inclined to conclude that their action is identical and that likewise the immunity which they produce is the same; that animals having received preliminary injections of colloidal copper would therefore be immune not only against the action of colloidal copper but also against

hirudin, and that those having received preliminary injections of hirudin would also be immune against colloidal copper. We wished to test this conclusion and undertook therefore experiments in which we immunized animals with one substance and examined later their immunity not only against the substance with which they were immunized, but also against the other substance. These experiments showed that animals immunized with colloidal copper are essentially only immune against the effect of colloidal copper, not of hirudin, and those immunized with hirudin are immune against hirudin, but not noticeably (very weakly, if at all) against colloidal copper. The acquired immunity is therefore a specific one. This specificity can be shown to exist if after preliminary injections given from the second to the fifth day after inoculation the immunity is tested through cross injections given from the ninth to the thirteenth day. It can also be demonstrated in the tumors transplanted into other animals after preliminary injections in the first set of animals. The specificity concerns therefore the immunity which is produced in the tumor cells and probably also the immunity in the organism of the injected animals. These investigations prove then (1) that an acquired immunity against the injurious action of certain substances can be localized in the cells concerned; (2) that this immunity can be transferred to later cell generations; and (3) that although the effect of two substances on the cells is apparently the same, the mechanism through which this effect is produced differs in the case of each substance, and that therefore the immunity produced against the injurious action of these substances is a specific one for the substances injected. We see therefore that in a similar manner as in germ cells an effect produced through an external agency can be transmitted to later generations; a transmission of changes produced through an external (chemical) agency may be transmitted to later generations also in the case of somatic cells. But the further results we obtained in the case of somatic cells suggest the question whether the lesion produced and transmitted

in germ cells may not also be specific, although different chemicals produce apparently the same results. May there not in germ cells, just as in somatic cells, exist a difference in the mode of production of these lesions through the different substances and consequently a specificity in the acquired lesion, notwithstanding the apparently unspecific character of the lesion? Our work makes it possible that this question which seems of considerable theoretical interest may be solved in a similar manner as in the case of the somatic cells, viz., through testing the immunity produced through the action of chemical substances.

Summary

1. It is shown that evidence similar to that which makes probable the potential immortality of protozoa and germ cells also exists in the case of somatic cells of metazoa. As far as the protozoa are concerned, the discussion does not, as seems to be assumed, concern their potential immortality so much as the question whether protozoa correspond to germ or to somatic cells of metazoa or represent perhaps a combination of both.

2. While in the case of tumor cells the potential immortality of somatic cells has been demonstrated as definitely as the character of the problem will ever permit, the difficulties standing in the way of a similar demonstration in the case of certain other somatic cells and the means of overcoming these difficulties are analyzed. It is particularly shown that chemical differences existing between the body fluids of individuals belonging to the same species are the basis of these difficulties, and that as a result of these differences the metabolism of cells in a new environment is modified in such a way that the behavior of connective tissue cells and of lymphocytes is altered, and that as a result of these alterations the death of the tissue is brought about where it could probably have lived indefinitely. Besides altering the character of the body fluids, transplantation of tissues furthermore has usually an additional injurious effect; it changes the way in which nourishment is carried to the transplanted cells, and this

may also lead to alterations in metabolism calling forth a destructive activity of connective tissue and lymphocytes. Thus there exist difficulties in the case of certain tissues in demonstrating through serial transplantation their potential immortality, in a similar manner as it has been demonstrated in the case of strongly proliferating somatic cells (tumor cells).

It is also shown that in the case of the germ cells which really represent a foreign individual within a host organism mechanisms exist which prevent these injurious agencies from becoming effective.

3. We must sharply distinguish between the power of cells to grow and their power to live. While the former seems to be destroyed through differentiation, the latter may still exist, and we can therefore at present not deny the possibility that even highly differentiated somatic cells may still possess the potentiality of immortal life.

4. While in the case of protozoa and germ cells definite cycles exist, manifesting themselves either through the occurrence of rhythmically occurring depressions of vitality or of typical changes in the nuclei, such cycles have so far not been demonstrated in the case of somatic cells, particularly of tumor cells.

5. In the case of germ cells external factors can produce certain changes, and these changes (not necessarily identical with those originally produced through the external factors) can be transmitted to the offspring. It is shown that in a similar way in the case of somatic (tumor) cells a transmission of characters acquired under the influence of external agencies to the succeeding cell generations may take place. It can be shown in the case of the somatic cells that apparently similar changes produced through different external agencies are really not identical, but specific. It is suggested that such a specificity of transmitted characters may also exist in the case of germ cells despite the apparent identity of changes produced through different external agencies.

MENDELIAN INHERITANCE OF FECUNDITY IN THE DOMESTIC FOWL, AND AVERAGE FLOCK PRODUCTION [1]

DR. RAYMOND PEARL

In 1912 I showed,[2] from extensive experimental data that, in certain breeds of domestic poultry, winter egg producing ability is inherited in a strictly Mendelian manner. It was pointed out that there was much evidence indicating that winter production was, on the whole, a rather reliable index of total fecundity capacity. As was to be expected, the novelty of the results presented in the papers referred to led to their criticism from various points of view, including that of the practical poultryman. Most of these criticisms have been based upon some misunderstanding of the nature of the results themselves. Others, and particularly those of the poultry press, have apparently been based on a purely conservative instinct to resist the intrusion of any new idea which seems to threaten those solid personal and editorial assets of (reputed) infallibility and "safe and sane" judgment.

It has seemed to the writer more likely to conduce to the advancement of knowledge in this field if he went steadily about collecting more and more concrete objective evidence rather than engaging in polemic disputations with everyone whose opinion in regard to the validity or interpretation of the earlier results chanced to differ from his own. As a result of this policy there has accumulated a large mass of additional experimental data confirming and extending the results of the earlier work.

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 81.

[2] Pearl, R., "The Mode of Inheritance of Fecundity in the Domestic Fowl," *Jour. Exper. Zool.*, Vol. 13, pp. 153–268, 1912. *Cf.* also "The Mendelian Inheritance of Fecundity in the Domestic Fowl," AMER. NAT., Vol. XLVI, pp. 697–711, 1912.

This material will be published as opportunity offers.

It is the purpose of the present paper to record certain facts which are pertinent to a general consideration of the problem of inheritance of fecundity, but at the same time do not fall in the direct line of the experimental inquiry. They are matters, in other words, which are essentially by-products of the investigation but still have a more or less important bearing on the interpretation, in a broad sense, of the whole.

I. The Seasonal Distribution of a Flock Egg Production under a Mendelian System of Breeding as compared with Simple Mass Selection

The mean egg production per bird in the different months of the laying year has been given by Pearl and Surface[3] in an earlier paper. Those results are based on the weighted mean production of the flocks of Barred Plymouth Rocks at the Maine Agricultural Experiment Station during the ten years that a system of mass-selection was followed in breeding for egg production.

It is an obvious deduction from the results of the Mendelian experiments recorded in the earlier papers already referred to, that by their application it should be possible to modify the average production of a flock over a rather wide range, the modification being of a fixed and permanent character under any definite conditions of environment and breeding. To many practical poultrymen the only test of the validity of the conclusions reached which has any significance, is that of average flock production. It is obvious that from a technically critical point of view such a test has, of itself, relatively small value in helping to judge of the correctness of a Mendelian interpretation. At the same time it is clear that if one takes a flock of poultry of mixed genetic constitution in respect of fecundity and aims to preserve in his breeding only animals carrying both the factors L_1 and L_2 necessary for high

[3] Pearl, R., and Surface, F. M., "A Biometrical Study of Egg Production in the Domestic Fowl." II. Seasonal Distribution of Egg Production," U. S. Dept. of Agr., B. A. I. Bull. 110, Pt. II, pp. 81–170, 1911.

production, there ought to result a marked and immediate improvement in average flock production no matter what the size of the flock.

This, as a matter of fact, is exactly what has been done in the breeding of the flock of Barred Plymouth Rocks at the Maine Station for several years past. No attempt has been made to propagate low fecundity strains, after it had once been demonstrated that this could be done. In the work since 1912 the experimental aims have been such as not to be at variance with the practical one of getting the most eggs with the least trouble and expense, so far as has concerned the Barred Plymouth Rock stock. Consequently in making the matings from which the foundation Barred Plymouth Rock stock was being maintained I have each year endeavored to keep a number of different blood lines comparatively pure for the factors L_1 and L_2, and then intercross these lines with one another.

The results have been highly successful from a practical point of view. This is indicated by the figures shown in Table I and graphically in Fig. 1. These compare the mean egg production per bird month by month under the old system of mass-selection and under the new system of breeding which recognizes the Mendelian inheritance of fecundity with sex-linkage of the factor on which high production depends. The figures for the new system are those of the laying year 1913–14. In the laying year 1912–13 the flock had not yet attained any considerable degree of homogeneity in respect of fecundity factors since up to and including the preceding year low producing genetic combinations had been deliberately propagated and therefore an average which included all birds in the flock would be manifestly unfair as a test of the practical worth on a large scale of the new systems of breeding. The laying year 1913–14 is then the first completed year on which records are available for a fair test of the Mendelian plan on a total flock scale.

The Barred Rock flock of the year 1913–14 included 192 birds which completed the year's work. A number of other birds (about 20) began the year but died before its

completion. These 192 birds were divided among three flocks of 125 each, the other birds in each flock being cross-breds of various sorts.

It is possible to compare these 1913–14 flock with the old records during nine months of the year only. The reason for this is found in the fact that the trap-nesting season is, under the present system of management, brought to a close with August. Furthermore a record is now kept of the laying of the pullets in October at the beginning of the year, whereas formerly the season's records did not begin until November 1. This comparison is made in Table I. Also in this table the production for 1913–14 is compared with the *best* single year during the mass selection experiment, when anything approaching a corresponding number of birds were included,[4] and for which all environmental conditions may be regarded as approximately normal.[5] The single year records which come nearest to fulfilling all the conditions for a fair comparison with 1913–14 are those for the 100-bird pens in the laying year 1905–06. There were two such pens and 182 birds survived through the year. There was one small environmental accident in that year which reduced the production in May somewhat.[6] There were adverse environmental influences in 1913 probably quite as effective in reducing production as anything that operated in 1905–06. The seasonal conditions, size of flock, etc., were all fairly closely comparable with those obtaining in 1913–14. At that time (1905–06) the flock had been under continuous mass selection for eight years.

There are a number of difficulties in the way of making a comparison between any single year now, and the "best

[4] The absolutely best single year under mass selection was 1901–02. That year there were only 48 birds for which records are available. These were in several respects a special lot, and can not fairly be compared with large flocks kept under ordinary flock conditions. Cf. Pearl and Surface, *loc cit.*

[5] *Cf.* Pearl, R., and Surface, F. M., "A Biometrical Study of Egg Production in the Domestic Fowl. I. Variation in Annual Egg Production," U. S. Dept. Agr., B. A. I. Bull. 110, Pt. I, pp. 1–80, 1909, for an account of the environmental difficulties in certain of the earlier years.

[6] See Pearl and Surface, *loc. cit.*, p. 18.

year'' made under the mass-selection system. In the first place in order to make the comparison at all fair the flocks in which the birds were kept when the records were made must be of approximately the same size. It has been conclusively demonstrated by earlier work in the laboratory that egg production becomes reduced as flock size increases. It would be idle to compare the results now where the birds run in flocks of 125 to 150 birds per pen with the ''best'' of those prior to 1904, when the flocks were never larger than 50 birds each and were sometimes smaller. This restricts single year comparisons then to the period after 1904.

In the second place, if we take the year when the total production was highest, as the ''best'' year, we shall find, in practically every case, that some particular month or months of this year will fall below the average for that month or months. There are then two alternatives, either, on the one hand, to take for comparison with a single year now that year under the old system of breeding which, on the whole, is the best and then make allowances for disturbing factors in particular months, or, on the other hand, to compare a single year now, month by month, with an artificial year's record made up by picking out the best record of each individual month regardless of the year in which it occurred or of the size of flock. The second of these comparisons is obviously artificial, since it is continued high production month after month in the *same* laying year which is important. It is of interest, however, to see the results of the comparison on both bases. These comparisons are made in Table I.

The best single year 100-bird pen record in the earlier period is, as already pointed out, that for 1905-06, having regard to the months here compared (November to July, inclusive). The 100-bird pen of 1904–05 made a better record during the summer than the corresponding pens of 1905–06, but fall considerably below in winter production. In 1905–06 there was an environmental accident

TABLE I

Monthly Distribution of Mean Egg Production per Bird under Different Breeding Systems

Month	Weighted Mean Under Mass Selection	Best Comparable Year to 1913–14 of Similar-sized Flocks Under Mass Selection (1905–06 100-bird Pens)	Best Month in Any Year of Mass Selection, Any Size Flock	Year 1913–14
November.	4.63	5.38	6.45 (1904–05, 100-bird flock)	*10.76*
December .	8.91	9.91	12.02 (1901–02, only 48 birds in small flocks)	*14.19*
January....	11.71	13.27	15.21 (1901–02, only 48 birds in small flocks)	*13.88*
February..	10.87	13.39	14.46 (1905–06, 50-bird flocks)	*13.37*
March....	16.11	17.33	18.29 (1905–06, 50-bird flocks)	*19.22*
April.....	15.85	16.48[7]	18.50 (1901–02, only 48 birds in small flocks)	*18.44*
May......	13.92	——[8]	17.02 (1902–03, 147 birds in small flocks)	*16.88*
June......	12.46	13.47[7]	16.88 (1901–02, only 48 birds in small flocks)	*14.56*
July......	10.87	10.49[8]	14.90 (1901–02, only 48 birds in small flocks)	*14.52*

(overfeeding of green food) in the latter part of April. This adversely affected the May production. The 100-bird pens were more affected than the 50-bird pens. Consequently, in order to give every possible advantage to the earlier period of the work, I have taken the 50-bird pen averages for April, June and July and have graphically interpolated the figure for May in the diagram.

The data in Table I are set forth graphically in Fig. 1. From the table and the diagram the following points are to be noted:

1. It is apparent that the laying in the part of the laying year covered by the statistics was distinctly better in 1913–14 than either the weighted mean of the whole period of mass selection, or than in the best comparable year of the earlier period.

2. The difference is somewhat more pronounced in respect of winter production (*i. e.*, the laying prior to March 1) than for any other cycle. Under the earlier plan of breeding the average winter production was 36.12 eggs. This production corresponds reasonably closely to the division point at 30 eggs between genetically high and

[7] Average from 50-bird pens of same year (1905–06). See text.

[8] Average omitted because of abnormal conditions. See text.

genetically mediocre winter producers which was used in the Mendelian analysis. In the year 1905–06 the mean winter production was 41.95 eggs. In 1913–14 the pro-

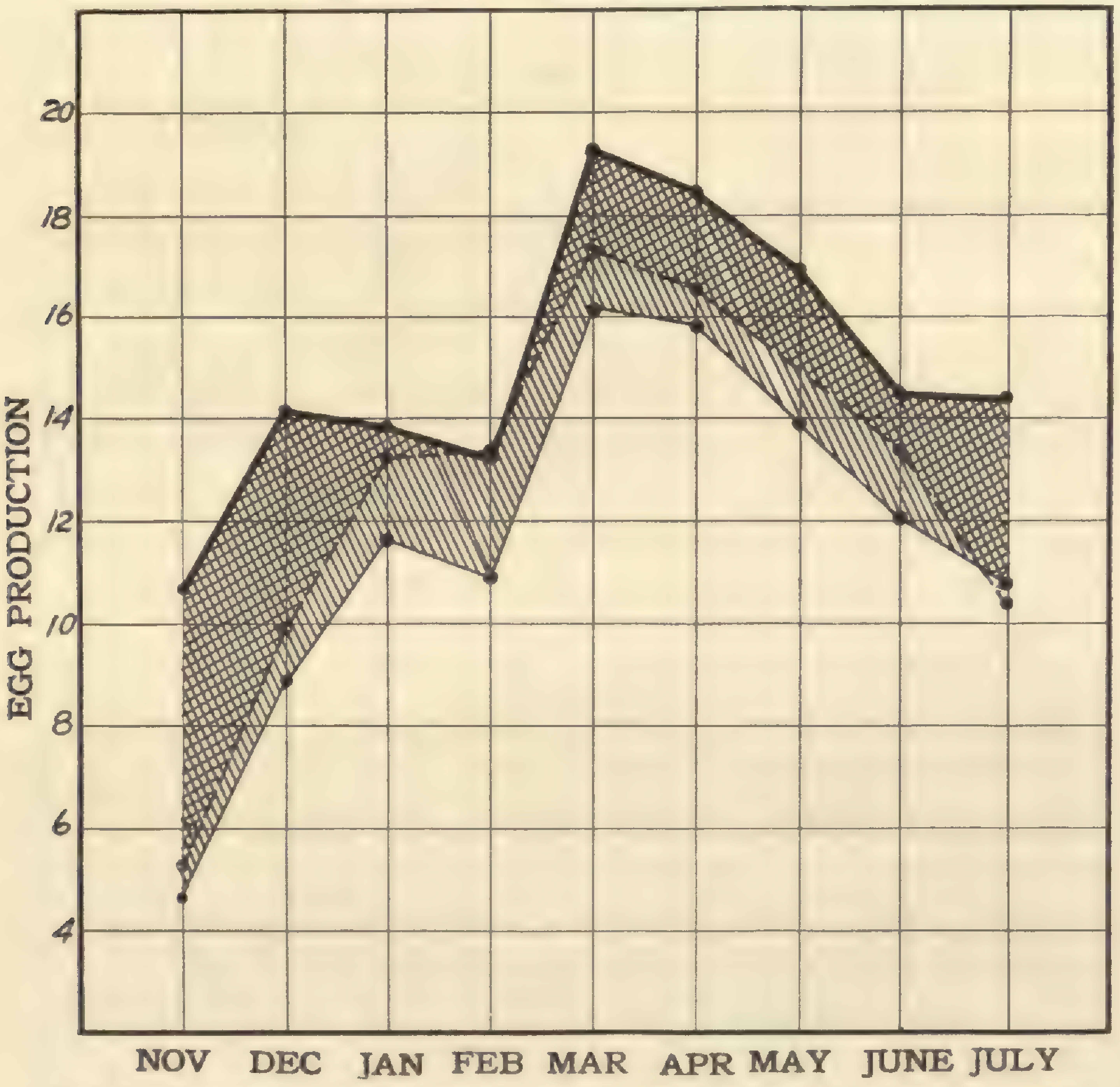

FIG. 1. Diagram comparing mean monthly egg production under different systems of breeding. The light continuous line gives the weighted means for the earlier years, the heavy continuous line the means for 1913–14, and the dotted line the means for 1905–06 100-bird pens. The cross-hatched area in comparison with the unruled area indicates in the increase of the 1913–14 averages over the earlier figures.

duction in the corresponding months was 51.20 eggs per bird.

3. It was shown by Pearl and Surface[9] that, on the average, a flock of hens produces 81.73 per cent. of their total annual yield between November 1 and August 1. Applying this figure to the 1913–14 nine-month total of 135.82 eggs, we get for the probable production of this

[9] "Biometrical Study of Egg Production in the Domestic Fowl. II. Seasonal Distribution of Egg Production," U. S. Dept. of Agr., B. A. I. Bull. 110, Pt. II, p. 89, 1911.

flock of 192 birds from November 1 to November 1 a total of 166.18 eggs. This value, as a matter of fact, is very close to the average production per bird of those (53) out of the 192 which were kept over for experimental purposes a second year. The corresponding total for the weighted mean annual production over the whole period is 128.86.

4. Taking the artificial year given in next to the last column of the table it is seen that in 1913–14, with 125-bird flocks, the November, December and March averages were higher than the highest made in the corresponding months during the mass-selection period, regardless of size of flock or other conditions. The April, May and July averages in 1913–14 were substantially equal to the highest made in the corresponding months under mass-selection. The highest January, February and June averages in the mass-selection period were from 1 to 2 eggs higher than the corresponding months in 1913–14. Taking the totals of the whole 9-month period compared, we have for the artificial year, made up of the highest mean monthly production under mass selection for each month regardless of the year or the flock size, *a total of 133.73 eggs per bird, while that for the single year 1913–14 is 135.82.*

Another comparison, which brings out some additional facts, is set forth in Table II. Any bird laying 18 or more eggs per month in the months November, December, January and February may certainly be regarded as a high winter producer. The proportion of such high producers in the whole flock gives valuable additional information to that furnished by the means, since the monthly egg production variation curves are distinctly skew. The

TABLE II

SHOWING PROPORTION OF FLOCK LAYING 18 OR MORE EGGS IN THE SPECIFIED MONTHS

Month	Total Flocks 1899–1907, Per Cent.	100-bird Flocks 1905–1906, Per Cent.	Flock of 1913–1914, Per Cent.
November	7.0	5.5	26.0
December	19.0	30.2	47.4
January	24.2	36.3	42.2
February	22.6	36.3	31.8

mean and median do not coincide. In Table II is shown the percentage of the whole flock laying 18 or more eggs in the months specified.

This table shows in an even more striking way than the means in Table I the marked difference between the flocks of the present time and those of the earlier years. In 1913–14 nearly half the flock laid 18 or more eggs each during December and January.

The data presented in this paper establish, I think, the following facts:

1. There is a marked difference in the average production per bird of Barred Plymouth Rock pullets of the Maine Station strain at the present time, as compared with what obtained in the earlier trap-nesting work of the Station described by Pearl and Surface (*loc. cit.*).

2. This difference is in the direction of *a substantially higher mean flock production at the present time.*

3. The increase in flock production is most pronounced in respect to winter production.

The most probable explanation of the above results appears to the writer to be that the plan of breeding now followed is more nearly in accord with the biological facts regarding the inheritance of fecundity than was the plan followed in the earlier years.

The reasons for this opinion, while not constituting complete proof of the suggested explanation, certainly make a strong body of evidence in its favor. They are, summarily stated:

(*a*) That the increases in flock productivity have been synchronous with changes in breeding practise.

(*b*) That the increases give every indication of being permanent, there having been no tendency towards a decline in flock productivity since 1908, when the simple mass selection was stopped and breeding begun on a progeny-test basis.

(*c*) That there have been no changes in management or environmental circumstances synchronous with the increases in flock production and capable of accounting for them. The hens are housed to-day in the same houses

that they were in 1904; are fed substantially the same feed, the only modification of the ration having been in the direction of one *less* stimulating to production than the one formerly used; are hatched in the same sort of incubators; reared in the same yards, etc.

(*d*) That the most marked gains have been in that cycle of production (winter laying) to which especial attention was paid in the breeding.

(*e*) That when analyzed in terms of individual matings the results obtained in egg production have been the results to be expected on the Mendelian hypothesis of the inheritance of this character earlier set forth, with only minor exceptions for which the explanation is in nearly all cases apparent.

II. An Independent Confirmation of the Sex-linkage of the Factor for High Fecundity

Besides the results with large flocks which have followed the practical application of the Mendelian hypothesis of fecundity inheritance at this Station, numerous poultrymen in various parts of the world have obtained similar results. Several instances of this sort might be cited from private correspondence. The writer has felt, however, that such cases really contributed nothing new in principle, and that therefore there was no special need of calling attention to them.

There lately appeared, however, in an English poultry paper, a note which seemed to me to be of interest on several grounds. In the first place, it is evident that the writer, Mr. E. N. Steane, is a careful observer, and an experienced poultryman. In the second place, his observations on inheritance of egg producing ability appear to be, from his point of view, entirely original and uninfluenced by any earlier work.

The parts of Mr. Steane's note[10] which are pertinent in the present connection are these:

[10] Steane, E. N., "The Production of 'Best Layers,'" *The Feathered World* (London), Vol. 52, p. 285, 1915.

My own experience, and that of many other breeders, tends to show that the birds hatched from high pedigree hens are not such prolific layers as those hatched from healthy hens of an indifferent laying strain mated to high pedigree cockerels.

For three or four seasons I bred from two-year-old white Leghorn hens of a gold-medal laying strain mated to a cockerel of equally-good descent, and the results, to my mind, were disappointing, and did not yield an adequate profit on the money spent. The pullets were less prolific than their parents, and inclined to be delicate and more or less undersized, while the percentage of fertile eggs was lessened.

Then by a lucky chance one season I had not enough eggs from a pen of Rhode Island Reds to fill up an incubator, and I made up the deficiency from a pen of good-sized healthy Leghorn hens of no particular laying strain mated to a pedigree cockerel. Practically every egg from this pen was fertile, the chickens proved strong, and the results seemed in every way satisfactory.

This, of course, led to my systematic mating of healthy, well-grown birds of indifferent laying strain to high pedigree cockerels, with very successful results. The fertility of the eggs was extremely satisfactory, the chickens turned out strong and healthy, and the pullets on arriving at maturity were highly prolific layers, each pullet averaging 200 eggs and over during the first twelve months, as against about 130 from the pullets of the high pedigree hens, many of whom also died off. In the second year the birds did equally well, the number of eggs being maintained and all being of a good size.

Later, I tried the result of mating high pedigree hens to a healthy cockerel of no special laying strain, but without success, the chickens being healthy, but the laying results much below the average, so that nothing was to be gained by further trials in that direction.

While being quite aware that many breeders do not agree with my conclusions, and that a great deal also depends on the condition and environment of the birds—prolificacy being always greatly improved by the birds having a free range, I am myself firmly convinced that such mating makes for the production of best layers. All my experiments were, of course, carried out under the same conditions in each case, the birds being kept in runs of 20 yards by 10, on well-drained, sandy soil, with a house and scratching shed attached, and fed on the same diet as that adopted in the recent laying competitions.

It is evident that Mr. Steane's experience was exactly parallel to the results of the present writer's investigations reported in earlier papers. High producing females did not transmit that quality directly to their daughters. The character is sex-linked.

The only point of difference is that noted in the second

paragraph of the quotation, and I think that the explanation of the discrepancy there is contained in the closing words of the paragraph where Mr. Steane says:

> The pullets were . . . inclined to be delicate and more or less undersized, while the percentage of fertile eggs was lessened.

This would indicate that other causes besides the breeding operations were working to bring about a poor physiological condition of the progeny, which is of course inconsistent with high productivity. Lowered fertility of eggs is one of the best indicators of reduced vitality which can be found.

We appear to have, in this case, a rather complete independent confirmation by a practical poultryman of one of the present writer's chief results in regard to the inheritance of fecundity.

III. Summary

In this paper it has been shown that:

1. There is a marked difference in average egg production per bird of Barred Plymouth Rock pullets of the Maine Station strain at the present time as compared with what obtained during the period of simple mass-selection for this character.

2. This difference is in the direction of a substantially higher mean production at the present time, when tested on flocks of large size.

3. The increase in flock average productivity is most pronounced in respect to winter production, which is the laying cycle to which especial attention has been given in the breeding.

4. The cause of this increase in flock productivity appears, with a degree of probability which is very high and amounts nearly to certainty, to be that the method of breeding the stock now followed is more closely in accord with the mode of inheritance of fecundity than was the simple mass-selection practised in the earlier period.

5. The result announced in earlier papers that high fecundity is a sex-linked character, for which the female is heterozygous, has been confirmed by practical poultrymen in their breeding operations.

SHORTER ARTICLES AND DISCUSSION

THE APPEARANCE OF KNOWN MUTATIONS IN OTHER MUTANT STOCKS

In *Drosophila ampelophila* the reappearance of known mutations in stocks that appear to be uncontaminated is a not unfamiliar occurrence, but we discount all such cases unless in some way the occurrence can be controlled, because the chance of contamination even with extreme care might be claimed to be greater than the chance of mutating.

In the stock of sepia-eyed flies a few individuals with very pale (yellowish red) eyes appeared. Sepia eyes are very dark or black brown in color. So that the flies with the new eye color stood out conspicuously amongst the dark-eyed sepias.

From the color of the eye it was suggested that it might be vermilion-sepia. If this were the case it should give, when bred to vermilion flies, vermilion-eyed offspring, because the factor for vermilion would be common to both stocks and the stock vermilion would carry the normal (dominant) allelomorph of sepia. When the test was made the offspring were vermilion. These F_1's inbred gave in F_2 122 vermilion to 39 vermilion sepia, approximately 3:1, which is the expectation for one factor difference. The result shows that a mutation to vermilion eyes had taken place in stock that had already sepia eyes. The resulting flies were the double recessive vermilion sepia.

That the result is not due to contamination is evident, for had a vermilion-eyed fly got into the sepia stock it would have produced red-eyed (wild type) females or vermilion males. As no red- or vermilion-eyed flies were present this explanation is excluded.

A similar mutation took place in stock having purple eyes. Like sepia the eye color of these flies is dark but in this case has a distinct purplish-red tinge. Among the offspring from a cross of a female heterozygous for purple with a pure purple male a fly with very pale orange-colored eyes appeared. This fly, which was a male, was also conspicuously unlike the remainder of the red or purple offspring of this pair.

It was at first thought possible that this was the appearance of a mutation to cherry in a mutant stock. If this had been the case we should expect that in a cross to a cherry female all of F_1 offspring would be cherry. The test showed instead all cherry

males and all red females—the normal dominant color. This proved then that the new fly did not contain the factor for cherry.

It was then suggested that this was a second case of the appearance of vermilion in mutant stock. Since the fly was a male several matings were possible, and it was therefore crossed to a vermilion female. As in the previous case if vermilion were common to both stocks, the offspring should be all vermilion. This condition was actually found in all the F_1 offspring of this second cross, and the F_2's gave vermilion and the orange-eyed fly—now shown to be vermilion purple—in approximately 3:1 classes. This demonstrated that a mutation to vermilion had taken place in a fly already having purple eyes, for as the cherry cross indicated, the pure mutant stock contains the normal dominant allelomorph to every factor except the one it shows.

In order to demonstrate that the purple factor was still present unchanged in the germ cells of the double recessive (vermilion purple) fly, the original male was also mated to pure purple-eyed stock. As in the other cases if both parents contain the factor for purple all the offspring should be purple. This was the actual result obtained, and it proves the original mutant to have been the double recessive vermilion purple.

In this case also it is not possible that the result could have been due to contamination. This would indeed be highly improbable when the original parents were a single isolated pair, the female of which was a virgin when first mated. But even had a vermilion male been able to mate with the heterozygous female only red-eyed flies could have been produced. The vermilion purple combination could not occur because the germ cells of each animal carry the normal dominant allelomorph of the mutation in the other.

Our results in these two cases show that mutations within other mutant stocks occur, and they also indicate that in the case of vermilion we have a mutation which has recently reappeared twice.

T. H. Morgan,
Harold Plough
Columbia University

THE EVENING PRIMROSE VARIETIES OF DE VRIES

No explanation of the "variation" of heterozygous plants had presented itself until Mendel went back to the haploid generation, and referred the differences in the progeny of heterozygotes to the segregation of differences in the pollen-grains and embryo-sacs from which the plants had arisen. He dealt, however, with plants

in which all the young pollen-grains and embryo-sacs had presumably an equal chance to mature.

Perhaps a parallel state of affairs exists to-day. De Vries and others have brought to light, in the progeny of Œnotheras, a certain amount of "variation," by no means so striking as can be seen in the progeny of many variety or species crosses, but remarkable chiefly because it could not be explained. In my opinion, this "variation" can perhaps be explained by going back, as Mendel did, to the haploid generation. We may, I think, presume that there are certain genetic factors concerned with the development of the young microspore into a complete pollen-grain, and the young megaspore into a normal embryo-sac. If the plants are heterozygous for one or more of these factors, we get definite ratios of normal and aborted pollen-grains, or normal and aborted embryo-sacs. We thus have a population of haploid individuals (microspores or megaspores) which show segregation into viable and non-viable. We may expect ratios of normal and aborted haploid individuals of 1:1; 1:3; 1:7; 1:15, etc., applying to either pollen-grains or to embryo-sacs, or to both. When we thus have heterozygosity of one or more factors essential for the development of the individuals of the haploid generation, the laws and ratios for certain characters of the diploid generation may become very different.

According to Geerts, who seems to have made the only accurate study of the point, the typical *Œnothera lamarckiana* aborts one half its microspores (two from each tetrad) and one half its embryo-sacs. The simplest hypothesis demands two factors, one essential for the development of pollen-grains, and one for the development of embryo-sacs, which factors show complete (or nearly complete) repulsion. Then, of course, the offspring will be permanently heterozygous for these two factors, and also more or less for any other factors which may be linked with them. (Linkage has been shown to exist, I think, in all plants where it has been looked for.) With more than two such factors heterozygous, the aborted pollen-grains or embryo-sacs may increase, and the ratios in the progenies of crosses become altered. Hence, in my opinion, a promising step towards the investigation of inheritance in Œnotheras is the correct determination of the ratios of aborted and normal pollen-grains and embryo-sacs in the different "varieties" of *Œnothera lamarckiana*, and related species.

JOHN BELLING

FLORIDA AGRICULTURAL EXPERIMENT STATION

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THE SIGNIFICANCE OF CERTAIN INTERNAL CONDITIONS OF THE ORGANISM IN ORGANIC EVOLUTION

First Paper. The Regulation of the Physico-Chemical Conditions of the Organism[1]

F. H. PIKE AND E. L. SCOTT

Department of Physiology, Columbia University

I. Introduction.
II. The general constancy of internal conditions in the higher organisms.
  1. Thermo-regulation in the higher organisms.
  2. The cardiac and vascular mechanisms.
  3. The respiratory mechanism.
  4. The concentration of sugar in the blood.
  5. The osmotic pressure of the body fluids.
  6. The digestive tract.
  7. The epidermis and other mechanical protective mechanisms.
  8. The internal secretions.
  9. The mechanisms of elimination.
III. The laws of chemical equilibrium.
  1. The law of mass action.
  2. Van't Hoff's law.
  3. The phase rule.
IV. The interpretation of the regulatory mechanisms in terms of chemical equilibrium.
  1. Applications of Van't Hoff's law.
  2. The general conditions of the reactions in the cells.
  3. Stimulation in terms of chemical equilibrium.
V. General considerations and summary.

The desirability of an attack on the general problems of evolution from the point of view of physiology, as well as the general deficiency of literature in physiology bear-

[1] Read before Section F of the American Association for the Advancement of Science, December 30, 1914.

ing on these problems, has been recognized for some time.[2] The considerations to be presented in the papers of this series are in part the outgrowth of experimental work on the central nervous system and its relation to the processes of evolution;[3] they were in part suggested by Blackman's[4] paper on the manifestations of the principles of chemical mechanics in living matter; and they have gradually grown up in our minds as our attention was attracted more and more to the questions involved. The bearing upon the processes of evolution of certain of the facts drawn from the experimental study of the comparative physiology of the nervous system will, for the most part, be presented in separate papers embodying the experimental data. The relation of the physico-chemical conditions of the organism, together with those nervous mechanisms with which the maintenance of the physico-chemical conditions is inextricably bound up, to the questions of adaptation and fitness of the organism will constitute the greater part of the subject matter of this and following papers on the general problems of evolution from the point of view of the physiologist.

Since the inception of the work, there have appeared, in addition to Blackman's paper, several other papers of interest to physiologists on certain phases of the problem of evolution.

Woods[5] has collected the better-known cases of modification in animals and plants induced by changes in the environment or in the general conditions of existence, as shown by changes in the rate of growth, changes of the external form of the body, the occurrence of artificial

[2] Howell, "Problems of Physiology of the Present Time," Congress of Arts and Sciences, Universal Exposition, St. Louis, 1904, Vol. V, p. 11 of the reprint.

[3] Pike, *American Journal of Physiology*, 1909, XXIV, p. 124; *Ibid.*, 1912, XXX, p. 436; *Quarterly Journal of Experimental Physiology*, 1913, VII, p. 1; *Popular Science Monthly*, 1914, p. 403; *Science*, N. S., XI, p. 805.

[4] Blackman, *Nature*, 1908, LXXVIII, p. 556; AMERICAN NATURALIST, 1908, XLII, pp. 633–664.

[5] Woods, *Popular Science Monthly*, 1910, p. 313.

parthenogenesis, the modification of mental and moral traits, and the extent of regeneration of lost parts. When arranged in the order of their position in the taxonomic scale, organisms show a steadily decreasing response, with reference to these phenomena, to changes in the environment. Woods does not indicate, except in very general terms, the physiological mechanisms involved in bringing about this diminishing effect of the environment. Some years earlier Donaldson[6] had called attention to the general lack of influence of formal educational training upon the course of later life of talented individuals.

Julian Huxley[7] in his discussion of the individual in the animal kingdom, points out that the individual acquires an increasing independence of the environment, or that the environment has a diminishing effect. Huxley assigns mere increase in size and the increasing complexity and efficiency of the nervous system as two of the factors involved in the attainment of the freedom from mere accidents.

Mathews[8] has pointed out more specifically some of the various internal mechanisms which are involved in the acquisition of independence of the environment on the part of the higher animals. These are, according to him, (1) the heat-regulating mechanism, (2) the mechanism of immunity, (3) the mechanism for rendering animals independent of external conditions of moisture, (4) the mechanism which renders them independent of barometric pressure, (5) the mechanisms for reproduction and caring for the young, (6) the alimentary mechanism, and (7) the nervous system.

Henderson[9] has shown that the environment is an external thing in which certain physico-chemical conditions are kept relatively constant, while others may vary

[6] Donaldson, "Growth of the Brain," London and New York, 1895, pp. 347, 355, 360, 365.

[7] Huxley, J. S., "The Individual in the Animal Kingdom," Cambridge and New York, 1912.

[8] Mathews, AMERICAN NATURALIST, 1913, XLVII, pp. 90–104.

[9] Henderson, "Fitness of the Environment," New York, 1913.

widely, even in the same region. As long as organisms live in the ocean or in the water generally, they are subjected to certain relatively constant conditions dependent upon the physico-chemical properties of the environment. But it should be recognized that with organisms which have migrated out upon the land the case is somewhat different, as all conditions, except oxygen concentration and pressure, vary more markedly on the land than in the water. Temperature, in particular, varies greatly over the land, and the poikilothermal animals have their activities greatly limited by the temperature conditions.

From Henderson's premises, the conclusion follows that life is what it is because the environment is what it is. A different environment might, and in all probability would, have resulted in, or been associated with, a different form of life on the earth. Certain characteristics commonly called adaptations are, as Henderson shows, the automatic and inevitable results of the physico-chemical conditions obtaining in the environment. If such a view assumes that an adaptation ceases to be an adaptation when the manner of its origin is discovered, it would seem that we were in need of a more precise definition of adaptation. The question arises whether other similar characteristics that have been called adaptations are not also the inevitable and automatic result of the physico-chemical conditions of the environment. We may therefore consider the question of the reality of adaptation, and also whether some of the characteristics of organisms are not really adaptations,[10] even if they arise from the action of physico-chemical conditions in the environment. Again, since, in a given environment, with essentially the same physico-chemical conditions for all its inhabitants, there are many animal types, it would appear that there were some influences operative within the organism itself to produce certain characteristic reactions, known as adaptations, to the environment. As we will show subsequently, these facts do not in any way preclude an explanation of their origin on some hypothesis

[10] Mathews, *loc. cit.*

other than vitalism. The organisms may be, under certain conditions at least, more variable than the environment. These considerations apply also to those simple organisms which are the cellular constituents of a larger body. Certain of the higher vertebrates, in which, as has been indicated, the influence of the environment is probably less than in some of the lower forms, are more variable than certain of the lower vertebrates.

It is a fair inference from the facts cited in the various papers referred to, that certain characteristics of living matter, such as its slight degree of alkalinity, and its relatively great specific heat, may be regarded as the direct automatic and inevitable results of the properties of the environment. Other characteristics, as the peculiar type of the nervous system, are not so obviously the direct and inevitable result of the environment, and these may be regarded, for some time to come, as adaptations to the environment or to the general conditions of existence in which the particular organism is able to live and perpetuate itself.

A statement of the general problem may now be made. Two general classes of organisms live in a relatively constant environment, so far as the general internal conditions of the organism are concerned. (1) For example, the lower marine organisms of fairly limited distribution live in an external environment which changes but little in temperature, osmotic pressure, inorganic salt content, neutrality or faint alkalinity, oxygen and carbon dioxide concentration, and soluble nitrogen compounds. Temperature and amounts of light may vary somewhat throughout the year, but the temperature changes in the given region of the ocean are less in magnitude than the temperature changes over a corresponding area of land. The osmotic and inorganic salt relationships of organism and environment alike vary but little. Nor does the organism, in general, maintain within itself, any purely physico-chemical condition differing greatly from that in the general external environment. This does not pre-

clude the origin within the marine organism of stereochemical isomers, the great importance of which has recently been pointed out by Reichert.[11] Stability of conditions would even favor the perpetuation of such compounds as could be formed under a given set of conditions. (2) On the land, as has already been indicated, the higher animals—birds and mammals—have acquired a relative independence of the environment as shown by their wide distribution. Their internal conditions of temperature, moisture and the like, may not only differ greatly from the same conditions in the environment at any given time, but the internal conditions, as we shall show, do not change greatly when the external conditions change. Intermediate between these two types is a third type which lives in an environment subject to wide variations of temperature and moisture, and whose internal temperature varies with the change of external temperature. While some of the internal conditions of these organisms remain relatively stable, others are subject to wide variation. We have then to explain, in terms of function, (1) what are the various mechanisms by which the higher animals have attained this relative independence of the environment and (2) what has been the rôle of these mechanisms in organic evolution.

II. The General Constancy of Internal Conditions in the Higher Organisms

The study of the internal physico-chemical relationships of higher animals has led to the acquisition of a considerable mass of facts concerning this phase of animal organization. These facts show that in the higher animals there exist a number of mechanisms which interact to bring about a remarkable constancy of internal physico-chemical conditions during the life of the animal. It appears justifiable again to call attention to some of these facts with a brief description of some of the mechanisms involved in order better to emphasize some of the interpretations of, and inferences drawn from, them in so far

[11] Reichert, *Science*, 1914, N. S., XI, pp. 649–661.

as these interpretations and inferences have to do with the general problem of evolution. We may, then, first of all briefly review the more salient of these facts, and afterward attempt their interpretation in terms of well-known physico-chemical laws—*e. g.,* the law of mass action and the phase rule.

1. *Thermo-Regulation in the Higher Organisms*

We may divide the vertebrates into two general groups on the basis of their internal or body temperature; (1) those which maintain a relatively constant internal temperature and (2) those whose internal temperature is variable. These groups may be tabulated as follows:[12]

TABLE I

I. Animals with a constant body temperature.	
Adults or partly grown mammals and birds	about 42° C. or 107° F. Birds. about 39° C. or 102° F. Mammals. about 37° C. or 98.6° F. Man.
II. Animals with an inconstant body temperature.	
(*a*) Animals which die when the temperature falls below 20° C.	Newborn mammals and birds.
(*b*) Animals which become torpid when the temperature falls below 20° C.	Hibernating forms—some mammals.
(*c*) Animals which remain active when the temperature falls below 20° C.	Reptiles, batrachians, fishes, among vertebrates; molluscs, insects, and the invertebrates generally.

Looking now to the differences between lower and higher vertebrates, to restrict ourselves at the start to a relatively small part of the animal kingdom, one of the most noticeable changes has been the development of a very constant body temperature in the so-called warm-blooded or homoiothermal animals (Table II). The detailed enumeration of the body temperature of all the animals so far observed would require too much space, but the following data will be sufficient to show upon what basis of fact the statements rest:

[12] Richet, Dictionnaire de Physiologie, 1898, t. III, pp. 85–86.

TABLE II

TABLES OF BODY TEMPERATURE

1. *Birds*

Genus or Species	No. of Observations	Temperature	Observer
Sparrow	1	42.1° C.	Davy.
Turkey	1	42.7	Davy.
Peacock	1	40.5 to 43.0	Davy.
Guinea fowl	1	43.0	Davy.
Duck, domestic	110	(Mean) 42.07	Martins.
Anas	179	(Mean) 42.3	Martins.
Anas		(Maximal) 43.45	Martins.
Anas		(Minimum) 40.8	Martins.
Palmipeds,			Davy, Eydoux,
divers	9	40.6	Brown-Sequard,
longipennes	69	40.6	Souleyet.
Pheasant	5	42.5	Richet.
Hen	17	42.5	Mantegazza, Demarquay, Dumeril, Davy, Prevost and Dumas.
Pigeon	600	(Mean) 41.9	Chossat.
		(Noon) 42.22	
		(Midnight) 41.48	
Pigeon	10	(Mean) 41.2	Corin, Van Beneden.
		(Maximum) 43.6	
		(Minimum) 39.	Daily variation 2.2
Pigeon	31	(Mean) 41.8	Zander.
		(Maximum) 44.	
General mean		42.0	

Richet[13] considers it probable that the temperature of birds is always above 40° and never, except under extreme conditions, above 44° C.

Further measurements are given by Sutherland Simpson.[14]

2. *Mammals*[15]

Genus or Species	Temperature
Pigs	39.7° C.
Sheep	39.6
Oxen	39.5
Rabbits	39.5
Dogs	39.2
Guinea-pigs	39.2
Monkeys	38.3
Horses	37.7
Monotremes	30.0

[13] Richet, *loc. cit.*, p. 87.

[14] *Proceedings of the Royal Society of Edinburgh*, 1911–12, XXXII, pp. 19–35.

[15] Richet, *loc. cit.*, t. III, p. 91.

The monotremes occupy a peculiar position in the scale (Table III). Their internal temperature, while relatively constant and considerably above that of the external air, is notably lower than that of mammals generally.[16] This group probably presents a transition stage between the poikilothermal animals and the homoiothermal animals with a higher body temperature.

TABLE III

SHOWING BODY TEMPERATURE OF THE MONOTREMES

	Temperature		
	Cloacal	Peritoneal	External
Echidna aculeata, var. typ.	26.5° C.	29.0° C.	21.5° C.
Echidna aculeata, var. typ.	29.5	31.5	22.0
Echidna aculeata, var. typ.	30.5	31.5	18.0
Echidna aculeata, var. typ.	31.5	31.5	18.0
Echidna aculeata, var. typ. young	31.0	31.5	24.0
Echidna aculeata, var. typ. young	34.2	31.5	22.5
Echidna aculeata, var. typ.	34.0	36.0	31.5
Echidna aculeata, var. typ.	28.3	30.0	31.5
Echidna aculeata, var. typ.	28.3	26.9	20.0
Ornithorhynchus paradoxus	24.4	26.9	20.0
Ornithorhynchus paradoxus	25.2	25.2	23.0

The normal diurnal variation in temperature of the human has been studied by many observers. The temperature varies not only with the time of day, but with the taking of food, with age, with external temperature and with other conditions to which we will return later. On the average, the highest temperature is attained in the evening between five and eight o'clock, and the lowest in the early morning between two and six, with upper and lower limits of 37.5° C. (99.5° F.) and 36.3° C. (97.4° F.), respectively. The body temperature may, of course, be greater or less without any necessary implication of disease processes, but the figures given may be considered as fairly representative.[17] It seems well established that, in conditions of health, the daily variation in temperature is about one per cent. of the mean—98.4° F.—expressed in the Fahrenheit scale. It is a peculiar fact also that the

[16] Richet, p. 90, Table II, and C. J. Martin, *Philosophical Transactions of the Royal Society*, London, 1903, Series B, Vol. CXCV, p. 1.

[17] Stewart, "Manual of Physiology," 6th ed., New York, 1910, p. 605.

normal body temperature is fairly close to the upper limits of body temperature compatible with life. A variation of five per cent. from the mean, expressed in degrees Fahrenheit, is often cause for grave anxiety, and a variation of ten per cent. is usually fatal. Compared with this, the variation in body temperature of fishes which live in the water only a few degrees above the freezing point in the winter and in the tepid water of a shallow stream in the summer is enormous.

The highest temperatures recorded in the human, after which recovery has occurred, are shown in Table IV.

TABLE IV

Temperature	Disease Suffered	Observer
43° C.	Hystero-epilepsy	Miersejewski, cited by Lombroso.
42	Typhoid fever	Alvarenga.
42.3	Scarlatina	Bouveret.
43.6	Scarlatina	Vicente et Bloch.
42.2	Periostitis and pyemia	Weber.
43.3	Intermittent fever	Mader, cited by Seguin.
44	Intermittent fever	Hirtz.
44	Intermittent fever	Alvarenga.
44.6	Intermittent fever	Riess.
46	Intermittent fever	Bassanovitz.
46	Intermittent fever	Diez Obelar.
46	Intermittent fever	Capparelli, cited by Richet.
44.8	Intermittent fever	Whitney.
43.3	Rheumatism	Wilson Fox, cited by Seguin.
44.9	Hysterical icterus	Lorentzen.
44	Hysteria	Clemow, cited by Gilles de la Tourette.
45	Hysteria	Lombroso.
45	Hysteria	R. Visioli.
43.6	Hysteria	Sciamanna.
43.9	Fracture of the cervical vertebra	Brodie. Lorain.
44	Fracture of the 12th dorsal vertebra and delirium tremens	Simon.
43.8	Fracture of the 6th cervical (19 hours after traumatism)	Frerichs.

The somewhat alarming but relatively innocuous manifestations of hysteria are attended by a high temperature without the complicating factor of an infection, and pres-

ent, therefore, the effects of temperature alone. It will be noted that the body temperature attained in hysteria—45° C. or 113° F.—and 46° C. in a case of intermittent fever with recovery is nearly as high as we have found recorded—45.7° C. or 114.3° F. after death in a case of tetanus, and is next to the highest recorded, as far as our observation goes, with recovery of the patient. The temperature of the body may, however, rise still higher under the influence of external agents. Thus, in death from strong electric currents, Klein[18] reports a body temperature of 132° Fahrenheit (55.5° C.) or even 140° F. (60° C.) immediately after death.

The effect of temperature upon the contractile manifestations of protoplasm has been summarized by Schäfer:[19]

> In warm-blooded animals the phenomena cease altogether to be exhibited if the protoplasm which is under observation is cooled to below a temperature of about 10° C., although they will be resumed on warming the preparation again, and this even if it has been cooled to 0° C., or a little lower. And when warmed gradually, it is found that the movements become more active as the temperature rises, attaining a maximum of activity a few degrees above the natural temperature of the body, although if maintained at an abnormally high temperature they are not long continued. A temperature a little above this maximum rapidly kills protoplasm, at least that of vertebrates, producing a stiffness or coagulation in it (heat-rigor), which is preceded by a general contraction; from this condition of rigor the protoplasm can not be recovered. But the protoplasm of some organisms will stand temperatures approaching that of boiling water without passing into heat-rigor. Freezing may cause destruction of protoplasm in higher animals, but that of certain of the lower animal and plant organisms is capable of resisting extreme cold, apparently for an indefinite time. This has also been found true for seeds of plants (Dewar).

Frog's muscle (gastrocnemius) reaches its maximum efficiency at about 35° C., after which a falling off occurs as the temperature is increased. Heat-rigor makes its appearance at about 41° C.—about two degrees Centigrade above the usual body temperature of a dog (an

[18] Klein, *New York Medical Journal*, May 30, 1914.

[19] Schäfer, "Text-Book of Microscopic Anatomy," London and New York, 1912, pp. 68–69.

average of 39.38° C. for 176 measurements), or slightly less than the usual body temperature of birds (42° C.).[20]

The maintenance of a constant temperature is dependent not upon one mechanism alone, but upon the coordinated interaction of several mechanisms. The presence of a coat of fur or feathers has long been recognized as a factor in maintaining the constant temperatures of mammals and birds. The development of such a protective covering has many times been emphasized by evolutionists, and seems to be well accounted for on the theory of natural selection. The fur or feathers tend to diminish heat loss from the surface of the body, but have nothing to do with the production or distribution of heat within the body. A subcutaneous layer of fat may still further reduce the heat loss from the surface, as in the Cetacea.

The production of heat is directly dependent upon oxidation in the muscles and glands of the body. A fall of general body temperature is attended by increased muscular activity, as shivering, when the temperature tends to fall unduly low. The muscular activity is dependent in its turn upon the nervous system, and upon the supply of oxygen and oxidizable substances through the blood.

The effect of the blood in maintaining a more nearly constant temperature of the muscles, as well as the production of heat by the muscles themselves, is shown by Meade Smith's experiments on mammalian muscles.[21] Although more heat is produced in a muscle which is contracting than in a resting muscle, there is still some heat production while at rest. When the artery going to a resting muscle was tied off, the difference in temperature between muscle and blood due to heat production in the muscle might be as much as 0.6° C. at the end of a five-minute period. When the circulation is intact, this difference in temperatures does not become so great. Tetanic stimulation of a muscle may lead to a considerable increase in the temperature of the venous blood coming

[20] Richet, "Dictionnaire de Physiologie," 1898, t. III, p. 86.

[21] Meade Smith, *Archiv für (Anatomie und) Physiologie* (Du Bois Reymond), Physiol. Abt., 1881, pp. 105–152.

directly from the muscle as compared with arterial blood.

The distribution of heat is accomplished by the circulating fluids of the body, and particularly by the blood. When the heat loss by radiation from the surface of the body becomes too rapid, the contraction of the walls of the peripheral blood vessels cuts down the quantity of blood going to the surface and, hence, the loss of heat as well. The constriction of the peripheral blood vessels and the contraction of the muscles tend to restrict the lower limit to which the body temperature may fall. The lower the external temperature, the greater the supply of heat from internal combustion needed to maintain the usual temperature of the body unless the radiation be checked by clothing or by artificial heat. It is generally stated that the temperature of the unclothed human body at rest may be maintained until the external temperature falls to 27° C. (Senator). This statement, as will be shown in a later paper, may be open to question. When the external temperature falls below this point, shivering or other involuntary muscular movement begins. This relation between temperature and metabolism accounts in large measure for the large amounts of food sometimes consumed by Eskimos. A young vigorous Eskimo may eat as much as four kilograms (nine pounds) of meat in a day.[22]

K. E. Ranke gives another illustration of the effect of climate upon diet in Germany and in Brazil. Allowing himself a free choice of food, the controlling influence being his appetite, his food requirements were 3,300 to 3,500 calories a day, when the external temperature range was from 15° C. to 22° C. In a dry atmosphere at 25° C., the fuel value of the diet fell to 2,800 calories. In an atmosphere with a temperature of 25° C. to 28° C. and a humidity of eighty-three per cent., the heat value of the diet fell to 1,970 calories in a day. This diet was insufficient

22 Rink, cited by Lusk, "Fundamental Basis of Nutrition," New Haven, 1914, p. 28.

to maintain his body weight, and disturbances of his general health appeared.[23]

The cold-blooded and warm-blooded animals react differently to changes in external temperature. Thus, the carbon dioxide output of a frog rose from 0.015 gram per kilogram of body weight per hour when the external temperature was 1.6° C. to 0.639 gram when the external temperature was increased to 34° C. (H. Schultz). But as was first shown by Pflüger and his pupils, the metabolism of a warm-blooded animal increases as the external temperature is lowered. If, however, the body temperature is raised there is likewise an increase in the metabolism of the warm-blooded animals. Pflüger regarded the increase in metabolism of the warm-blooded animals accompanying the decrease of external temperature as a later acquisition or as a mechanism which has gradually been evolved in the special interest of a constant temperature. Rubner's measurements of the metabolism in a dog showed an increase from 30.8 calories an hour when the external temperature was 27.4° C. to 40.6 calories an hour when the external temperature was lowered to 11.8° C.,—an increase of about thirty-three per cent. These considerations are sufficient to suggest that the effect of similar changes in the environment may not only not have effects of equal magnitude upon organisms at different levels in the taxonomic scale, but may even have opposite effects at the two extremes of the scale.

The upward march of the body temperature is restricted by the greater access of blood to the periphery and the increased loss of heat by radiation from the surface. A still greater loss of heat, in addition to the rather constant amount lost by evaporation of water from the lungs, is brought about by evaporation of water from the skin or other surface of the body, such as the tongue in dogs. The amount of water on the skin is regulated by the activity of the sweat glands, and these, in their turn, are under nervous control.

[23] Tigerstedt, "Text-Book of Physiology," translated by Murlin, New York, 1906, p. 407.

Animals unable to maintain this constant body temperature throughout the year may maintain a constant temperature during the warmer seasons of the year and hibernate during the winter. This is particularly the case with small mammals, whose relatively large ratio of surface to mass may greatly facilitate heat loss, and with animals whose supply of food is difficult or impossible to obtain during the colder season. The body temperature of the animal falls greatly during hibernation. The importance of size in its relation to metabolism may be shown from the specific energy requirements of various animals. In general, the heat requirement of all well-nourished warm-blooded animals is about 35 calories per hour for each square meter of body surface. But since the surface varies as the square of the dimensions of the body, and the mass varies as the cube of these dimensions, the ratio of surface to mass is much greater in small animals than in large. A mouse requires 452 calories per kilogram of body weight in twenty-four hours, while a horse requires but 14.5 calories and a man about 24 calories in the same time.[24] To sustain a number of mice equal in weight to a man would require more than eighteen times as much food, measured in calories, as a man would need; and more than thirty horses could subsist upon the same amount of food that would be necessary to sustain a number of mice whose aggregate weight was equal to that of one horse. This is very different from saying how much food one mouse as large as a man or a horse would need, and should not, under any conditions, be confused with such a statement.

Milne-Edwards observed that in a small bird such as the sparrow, the body temperature might be lower in winter—40.8° C.—than in summer,—43.77° C.—the difference in temperature amounting to about 3° C.

The heat regulating mechanism of the body is, then, not a simple one but a complex one, involving muscle and gland, food supply and distribution of the blood, the nerv-

[24] Lusk, *loc. cit.*, p. 10.

ous system and the oxygen tension in the blood. We may next consider some of these subsidiary mechanisms.

2. *The Cardiac and Vascular Mechanisms*

The mechanism for the distribution of the blood is in itself a complex one, and involves (1) the mechanism controlling the rate and force of the heart beat and (2) the mechanism controlling the caliber of the blood vessels. When the cardio-regulatory and vasomotor nervous mechanisms are intact, a fall in blood pressure is attended by an increase in the rate of the heart beat; and, conversely, when the blood pressure tends to rise, the rate of the heart decreases. When the extrinsic nerves to the heart are cut, these changes in the pulse rate no longer occur as an accompaniment to the changes in blood pressure. The importance to the animal of these changes in heart rate with changes in blood pressure is shown by the fact that rabbits and dogs whose extrinsic cardiac nerves have been cut soon get out of breath on attempting to run.[25] Through the combined agencies of the vasomotor and the cardio-regulatory nervous mechanisms, the blood pressure in all mammals so far investigated[26] and in some birds, *e. g.*, ducks and fowls,[27] is very much the same, that is, about equal to a pressure of one hundred and twenty-five millimeters of mercury. Changes in the caliber of the blood vessels or in the rate of the heart beat equalize the local changes of pressure due to changes in muscular activity. Working glands or muscles receive, as a rule, more blood than similar organs at rest. This increase in blood-supply may be due in part to the action of metabolites upon the walls of the blood vessels of the active structure (Barcroft).

The blood pressure in the homoiothermal animals, or the blood pressure during the periods of activity of such

[25] See Guthrie and Pike, *American Journal of Physiology*, 1907, XVIII, pp. 27–28, for the earlier literature.

[26] Porter and Richardson, *American Journal of Physiology*, 1908, XXIII, p. 131.

[27] Riddle and Matthews, *American Journal of Physiology*, 1907, XIX, p. 108.

of them as hibernate, is significantly higher than in the poikilothermal animals. The precise significance of this higher pressure in the homoiothermal animals is unknown. It has been suggested that a certain pressure is necessary to overcome the friction of the blood against the walls of the blood vessels. It would appear that fully as much friction might be encountered in the vessels of a turtle weighing thirty or more kilograms as in the vessels of a guinea-pig weighing less than one kilogram. Yet the guinea-pig has the higher blood pressure. Nor does the difference in blood pressure appear wholly due to mere differences in viscosity of the blood of the two forms.

The general stages, from the point of view of function, in the phylogenetic development of the vascular system have been indicated elsewhere.[28]

In connection with the question of the rôle of a more or less constant blood pressure in the animal economy, we may mention the experiments of Legallois, Schiff and Goltz.[29] These investigators found that, while the cells of an animal do not die immediately after the blood pressure reaches a low level, the life of the cells in such an animal as the frog is not possible for indefinite periods of time, and in rabbits or dogs, death is a matter of hours. We may, perhaps, imagine that the low blood pressure may give rise to changes in the chemical systems in the cell that are incompatible with indefinite existence.

3. *The Respiratory Mechanism*

The respiratory movements in mammals, and probably also in birds, are, as Haldane and Lorraine Smith have shown, kept up at such a rate as will maintain a constant tension of carbon dioxide and oxygen in the alveoli of the lungs, and presumably in the blood leaving the lungs. The oxygen and carbon dioxide content of arterial blood may be supposed to be fairly constant in any one indi-

[28] Pike, *Quarterly Journal of Experimental Physiology*, 1913, VII, p. 23.

[29] The literature on the effects of low blood pressure has been given in the *American Journal of Physiology*, 1912, XXX, pp. 444–446.

vidual. The reaction of the body fluids is likewise dependent, in some degree at least, upon the tension of oxygen and carbon dioxide in the blood.

The reaction of the body fluids, particularly the blood, remains remarkably constant during the life of the animal. This is not so much the peculiarity of the higher organisms as is the constant temperature. The process of regulation of neutrality, as Henderson has shown, is a physico-chemical process and depends upon the properties of carbon dioxide, bicarbonates and phosphates in solution. The changes in concentration of hydrogen and hydroxyl ions in the blood are, in their turn, related to the respiratory rhythm.

The whole subject has been so well summarized by Haldane[30] that I venture to quote his statement entire.

> To illustrate this point I may perhaps refer to a subject which we have recently been investigating at Oxford. We have found that the respiratory center is so extremely sensitive to any increase or diminution of the partial pressure of carbon dioxide in the blood that a diminution of 0.2 per cent. of an atmosphere, or 1.5 mm. of mercury will cause apnea, while a corresponding increase will double the breathing. The recent researches of Hasselbach have afforded experimental evidence of what had already seemed very probable—that the stimulus to which the center responds is the difference in hydrogen ion concentration, or acidity, brought about by the very slight deficiency or excess of carbon dioxide. He has also investigated quantitatively the effect on the hydrogen ion concentration of the blood of varying the partial pressure of carbon dioxide. From his results and ours it follows that the hydrogen ion concentration of the blood during rest is extraordinarily constant, and remains so day by day and year by year. As the amount of acid and alkali passing into the blood from the food and other sources is constantly varying, it follows that the regulation of hydrogen ion concentration is mainly brought about by the kidneys. It has been known for long that the urine varies in acidity or alkalinity according to the diet; but Hasselbach has measured the actual variations in hydrogen ion concentration. Putting together his conclusions and ours, it appears that during ordinary resting conditions the variations in hydrogen ion concentration of the urine are about a hundred thousand times as great as those of the arterial blood.
>
> Thus the kidney epithelial cells react so delicately to variations in

[30] Haldane, "Mechanism, Life and Personality," New York, 1914, pp. 49–51.

hydrogen ion concentration of the blood, that the very smallest variation in the direction of acidity or alkalinity excites them to excrete a liquid which is, relatively speaking, intensely acid or alkaline, the net result being that the normal hydrogen ion concentration of the blood remains practically constant.

When we have such figures before us we realize the marvellous fineness of the regulation by the kidneys and respiratory center. Physiologists are still so much under the influence of the old gross mechanical theories of secretion that attempts at exact measurements of the delicacy of regulation by the kidneys have hitherto scarcely been made in the case of regulation in other directions, though we have every reason to believe that similar delicacy exists as regards the regulation of the water, salts, and other blood constituents. It is hard to realize that something which looks under the microscope like nothing more than a somewhat indefinite collection of gelatinous material can react, and continue throughout life to react, true as the finest mechanism of highly tempered steel, to the minutest change in its environment.

TABLE V

Solubility of Gases in Water; Variation With the Temperature

The table gives the weight in grams of the gas which will be absorbed in 1000 grams of water when the partial pressure of the gas plus the vapor pressure of the liquid at the given temperature equals 760 mm.

Gas	8° C.	10° C.	20° C.	30° C.	40° C.	50° C.
O_2........	.0705	.0551	.0443	.0368	.0311	.0263
H_2........	.00192	.00174	.00160	.00147	.00138	.00129
N_2........	.0293	.0230	.0189	.0161	.0139	.0121
Br_2........	431.	248.	148.	94.	62.	40.
Cl_2........	———	9.97	7.29	5.72	4.59	3.93
CO_2........	3.35	2.32	1.69	1.26	0.97	0.76
H_2S........	7.10	5.30	3.98	———	———	———
NH_3........	987.	689.	535.	422.	———	———
SO_2........	228.	162.	113.	78.	54.	———

Whether such a constant O_2 and CO_2 content of the blood of all poikilothermal animals is to be found under all conditions of existence is unknown, but the coefficients of absorption of oxygen and carbon dioxide at varying temperatures would indicate that the carbon dioxide and oxygen held in solution in the body fluids would be subject to change with a change in temperature.[31] In the higher forms, the iron-bearing respiratory pigment, hemoglobin, absorbs all the oxygen with which it combines

[31] Table V, from Table No. 130, p. 142, Smithsonian Physical Tables.

within wide ranges of barometric pressure. There is some difference in the processes of absorption at high and low barometric pressures (Haldane), but the end result is essentially the same. No more oxygen is taken up by the hemoglobin at pressures of two or three atmospheres than at a pressure of half an atmosphere although the amount of oxygen dissolved in the fluid portion of the blood may be greater at the higher pressures. This difference is insignificant compared with the total oxygen in the blood. In the lower forms, manganese in the Echinoderms, and copper in certain Crustaceæ take the place of the iron in the respiratory pigment. The general conditions under which the oxygen is carried in the blood are, however, essentially the same in the various forms.

4. *The Concentration of Sugar in the Blood*

It has been stated since the time of Claude Bernard that the blood in the portal vein at the height of digestion contains more sugar in unit volume than the blood in the hepatic vein. The liver of the homoiothermal animals converts sugar to glycogen and stores it up in that form. Further extensive storage of glycogen occurs in the muscles.

While, under ordinary circumstances, there is good reason to believe that the concentration of sugar in the blood of a given animal is fairly constant for a given set of conditions, it is known that the amounts of sugar in the blood may vary under other conditions. Excessive amounts of sugar in the blood are eliminated by the kidneys. If the concentration of sugar falls, the transformation of glycogen to sugar makes up the deficiency. The concentration of sugar in the blood is not necessarily the same for any two species of animals under essentially the same conditions. Nor does the concentration of the sugar in the blood remain constant in any one individual, cat, dog, or human, under all conditions.[32]

[32] Cannon, *American Journal of Physiology*, 1914, XXXIII, p. 257; Scott, Ibid., 1914, XXXIV, p. 271; Shaffer, *Journal of Biological Chemistry*, 1914, XIX, p. 297.

It is probable that an organ or organs having a glycogenic function exist in some invertebrates, *e. g.*, the oyster, since glycogen may be obtained from these animals. Whether such a delicate adjustment similar to that obtaining in the higher animals between stored glycogen and circulating carbohydrate exists in these forms is unknown to us.

5. *The Osmotic Pressure of the Body Fluids*

In many species of homoiothermal animals, the osmotic pressure of the blood, as measured by the depression of the freezing point, is very constant, nor can it be easily influenced by changes in the environment. The ingestion of large quantities of water may be followed by the secretion of large quantities of dilute urine or by profuse perspiration. A shortage of water to drink leads to the secretion of small quantities of more concentrated urine. Both qualitatively and quantitatively, the physico-chemical constitution of the blood varies within relatively narrow limits.

6. *The Digestive Tract*

The mechanical and chemical processes of the alimentary tract reduce all the food, or its digestible portions, to water-soluble substances, in which form they are absorbed. The proteins of the food are reduced to the amino acids or polypeptids, the starches and sugars to monosaccharides and the fats to soaps before being absorbed.

The introduction of foreign proteins, *e. g.*, egg albumen, as such directly into the blood of the host is injurious. But the amino acids and polypeptids to which this protein is hydrolyzed by the action of the digestive enzymes are, in general, qualitatively the same as those in the blood of the host. The difference in proteins depends upon the difference in the quantitative relations of the amino acids in the protein molecule as well as upon the qualitative differences. The hydrolysis of all proteins to their

simpler splitting before absorption is a mechanism of protection against the entrance of foreign protein into the blood.

The alimentary tract to a certain extent renders the individual independent of the quantitative or stereo-chemical constitution of the proteins of its food, the only necessity being the presence of certain amino acids in the food.

But efficient as the alimentary tract is, it does not exclude all foreign or inutilizable substances. Cholohematin for example, from sheeps' bile, characterized by a peculiar spectrum and hence susceptible of easy identification, is absorbed by the dog and at least some of it is excreted in the dogs' bile without change.

The protection due to changes in the mucous membrane of the alimentary tract is well shown in the case of arsenic. It was long supposed that the arsenic-eating peasants of the Austrian Tyrol had become immune to the effects of a considerable concentration of arsenic in the blood, since they were able to take large quantities of it by mouth without suffering the usual effects of an overdose. It was shown by Cloetta[33] that the apparent immunity arose from changes in the intestinal mucosa which prevented the absorption of all but a small percentage of the arsenic taken.

If the lower members of the fatty acid series be fed to a dog the body fat which is stored up is softer and of a lower melting point than usual. Excess fat in the food may be stored up as body fat, but under certain conditions, the animal may use as much fat as is taken in the food, and the animal remains in metabolic equilibrium so far as the fat is concerned.

Although the amount of protein necessary to sustain life in an adult or to provide for growth in a young animal varies with the nature of the protein taken in the food, the animal is able to build up its own body protein from

[33] Cloetta, *Archiv für Experimentelle Pathologie und Pharmakologie*, 1906, LIV, p. 196.

any food which contains the requisite amino acids. The power of synthesis of new amino acids in the body is limited. Glycocoll, for example (amino-acetic acid), may be synthesized in the body. More protein is needed as food if its content in certain of the amino acids is low than if its content of these necessary acids is high.[34] But under all the varying conditions, within wide limits, the animal may maintain itself in nitrogen equilibrium, neither storing up any nitrogen in its flesh nor losing any from its tissues. The excess of any particular amino acid above that necessary for tissue growth or repair is eliminated from the body. Voit's famous 35 kilo dog was able to maintain nitrogen equilibrium when fed on amounts of protein varying from 480 grams of meat a day as a lower limit up to 2,500 grams a day.

The quantities of fluid, fat and protein in the blood, while undergoing some changes with the varying conditions of nutrition, starvation and fasting, remain close to a standard concentration as long as life lasts. Variations of great magnitude are incompatible with the prolonged life of the organism.

7. *Epidermis and Other Protective Mechanisms*

In addition to absorption, the limiting membranes of the body have other and important functions. The entrance of many harmful foreign substances into the organism is prevented by the protecting epidermis, the alveolar mucous membrane, and the intestinal mucosa, already noted.

But, however important the exclusion of harmful substances may be, the protection of the organism through the retention of valuable material is also of importance and must be provided for.

The escape of useful substances is prevented by the skin, the liver, the alimentary tract, the lungs and the kidneys. The splitting products of the hemoglobin arising from the death of the red blood cells are separated by

[34] Lusk, *loc. cit.*

the liver in the form of bile pigments, but the iron is conserved for use in the formation of new hemoglobin. But little iron is eliminated by the liver, although much iron may be stored there. The milk of higher animals contains but little iron, and the young mammal needs much iron for the formation of new blood cells during the rapid growth of early life. Provision is made for this by the storage of iron in the liver of the fetus. As postpartum growth proceeds, the amount of iron in the liver is reduced and the amount occurring in hemoglobin is increased.

8. *The Internal Secretions*

The internal secretions form another group of chemical conditions which vary within narrow limits in a state of health. Many years ago Caspar Friederich Wolff expressed the idea that each organ of the body stood in the relation of an organ of internal secretion to some other organ in the body. The liver liberates sugar which is necessary for the action of the muscles, and it also liberates urea which has a definite relation to the action of the kidneys. The muscles set free carbon dioxide which acts upon a particular group of cells in the nervous system, and nitrogenous waste products, some of which are transformed to urea in the liver. But apart from these more general relationships, a system of ductless glands sets free a variety of chemical substances which bear a rather different relation to the development and activity of many other organs and tissues of the body.[35] The relation of the adrenal gland to the myo-neural junction of sympathetic and smooth muscle fiber[36] is well known. The dependence of the development of the secondary sexual characters upon the internal secretion of the sexual glands is also familiar to biologists. The internal secretions in general have become a part of the internal conditions of the organism and of the chemical environment of the cells of the organism.

[35] Mathews, *Science*, 1897, N. S., V, pp. 683–685.

[36] Elliott, *Journal of Physiology*, 1905, XXXII, p. 401.

9. *The Mechanisms for Elimination of Waste Products*

The elimination of waste products occurs promptly and surely. The liver of higher animals not only separates out the useless portions of the hemoglobin derived from worn-out corpuscles (with retention of the iron-bearing part), in the form of bile pigment, but also transforms the ammonia compounds arising from nitrogenous metabolism to the relatively non-poisonous urea. The known chemical functions of the liver[37] are numerous. The kidneys promptly remove the urea and uric acid from the blood as these and other products of metabolism accumulate following activity of the cells. Certain mineral salts, *e. g.*, iron, are excreted by the intestinal mucosa. Some fat, in addition to that which is not absorbed from the food, is also excreted from the intestine.

The attempt to tabulate the various excretions of the body reveals the fact that, although the qualitative composition of the bile and the urine is relatively constant, the quantitative variations are very wide under different conditions. The quantity of carbon dioxide exhaled during twenty-four hours depends upon the diet and upon the amount of muscular activity during the day. Although somewhat disconcerting at first sight, in view of the constancy of internal conditions, this very inconstancy of the excretions is to be regarded as a consequence of the maintenance of constant internal conditions. For it is only by the conservation of those necessary elements or substances whose supply is limited, and the free elimination of waste products or of other substances in excess that the constancy of internal conditions can be maintained. Intake and outgo of matter and energy are such as to maintain relatively constant physico-chemical conditions within the organism. The greater the energy requirements of the organism, the greater must be the intake to meet these requirements, and the greater the amount of waste products eliminated.

[37] Hofmeister, "Chemische Organization der Zelle," ein Vortrag; Braunschweig, 1901.

The list of mechanisms, organs and tissues of the animal body and of specific organic or inorganic substances within the body, whose conditions and concentration remain relatively constant, could be extended. The description of these phenomena bulks large in the extensive literature of physiology of to-day. Sufficient data have been adduced to show that there is a considerable basis of fact for the interpretations which we now wish to present. A summary of the known facts of the internal conditions of the organisms permits of the statement that in the homoiothermal animals, there are, then, several mechanisms of extreme delicacy and great constancy under similar conditions and varying but little under wide changes of external conditions. The tracing out of their development constitutes a large part of the subject matter of comparative physiology. But their interpretation rather than their development is the thing of main interest at present. We will return to the questions of the origin and development of these mechanisms in later papers.

What point of view will give us the best insight into the rôle of these mechanisms in the evolution of the vertebrate phylum? Of what use have they been? Or are they simply mechanisms which have arisen in the course of evolution apparently through correlation with other phases of development but without obvious significance to the organism?

III. The Laws of Chemical Equilibrium

Before attempting the interpretation of these mechanisms, or pointing out their rôle in evolution, we may very briefly review the laws of chemical equilibrium as exemplified in the "slow" reactions of the physical chemist. For our present purpose, these laws may be included under the law of mass action, Van't Hoff's law and the phase rule. A fuller statement is given in Blackman's paper and in the text-books of physical chemistry.

1. *The Law of Mass Action*

The mass law or law of mass action expresses the relationship between the molecular concentration and the speed of the reaction. The concentration of the substance is usually expressed in terms of the number, either whole or partial, of gram molecular weights or gram molecules present in one liter of the solution. On the basis of Avogadro's law, there is the same number of molecules of sodium chloride in a gram molecule (58.4 grams) of sodium chloride as there are molecules of cane sugar in a gram molecule (342 grams) of cane sugar or molecules of oxygen in a gram molecule (32 grams) of oxygen. If equal fractions of the gram molecular weights of two different substances are each dissolved in a liter of water, there will be the same number of molecules in each of the two solutions.

Let two substances, *A* and *B*, be present in a solution in the concentrations (expressed in gram molecules) of c_A and c_B, respectively, at any stage of the reaction $A + B \rightarrow C + D$ and let the temperature remain constant throughout the action. The speed (S) of the forward action expressed in gram molecules of *A* and *B* transformed in unit time is defined by the relation $c_A \times c_B \times F = S$ where F is the affinity constant. As the reaction proceeds, c_A and c_B and, hence S, steadily decrease, since *A* and *B* are being continually used up. S may therefore be taken at any time as the quantity of *A* and *B* which would be transformed in the unit of time if the concentration c_A and c_B were maintained at a constant value by the continual addition of new substance. F is the measure of the intrinsic activity (affinity) which is the driving force in the reaction, and is independent of the concentration. If unit concentrations are taken, $c_A = c_B = 1$ and $F = S$. The activity, F, is thus represented numerically by the number of gram molecules transformed in unit of time when each reacting substance is present in unit concentra-

tion. Since c_A, c_B and S may be measured at any time, F may be calculated for any action.[38]

The law of molecular concentration or law of mass action is: In every chemical experiment, the speed of the action at any moment is proportional to the first, or some higher, power of the molecular concentration, at that time, of each interacting substance and to the intrinsic activity (affinity) of the substances.

2. *Van't Hoff's Law*

But the speed of any reaction at any concentration varies with the temperature. In general, the increase in speed is about ten per cent. for each increase of one degree Centigrade, or, as it is sometimes expressed, the speed of the reaction is doubled when the temperature is increased ten degrees Centigrade. This is known as Van't Hoff's law. The actual change in the speed of the reaction may be greater or less than ten per cent. for each change of one degree Centigrade, and is usually expressed by a coefficient. When the coefficient is 1.2 or less, that is, when the change in speed is two per cent. or less for each change of one degree Centigrade, the action is usually considered to be a physical and not a chemical action. When the temperature coefficient is greater than 1.2, the action is commonly considered to be a chemical action. No theoretical explanation of Van't Hoff's law of change in speed with change in temperature has so far been advanced.

These laws apply to reactions which go on at a measurable speed and which have been called "slow" reactions by the physical chemists. These "slow" reactions are to be distinguished from those reactions which proceed so rapidly that no measurement of their speed at different intervals is possible, or reactions of the explosive type.

[38] Smith, "General Inorganic Chemistry," 1st ed., New York, 1906, p. 251.

3. *The Phase Rule*

One other principle of physical chemistry finds frequent application in biology, and that is the phase rule developed by Gibbs. The phase rule defines the condition of equilibrium existing in a system by the relation between the number of coexisting phases and components. "According to it a system made up of n components in $n+2$ phases can only exist when pressure, temperature and composition have definite fixed values; a system of n components in $n+1$ phases can exist so long as only one of the factors varies and a system of n components in n phases can exist while two of the factors vary. In other words, the degree of freedom is expressed by the equation

$$P+F=C+2, \text{ or } F=C+2-P,$$

where P designates the number of phases, C the number of components, and F the degree of freedom."[39] In other words, F represents the number of conditions which may be varied without causing one of the phases to disappear.

An example of the phase rule, based upon the properties of a familiar substance, is that of ice, liquid water and water vapor existing together in a closed vessel from which the air has been exhausted. Ice, liquid water and water vapor each constitute a phase of the system, and there is but one component or substance—water—present. Here, one component exists in three different phases. We have, then, n components and $n+2$ phases. The essential conditions for the existence of the system are temperature and pressure of the water vapor. In the notation quoted above, $P=3$, $C=1$. Hence, $F=1+2-3=0$. Neither of the conditions—temperature or pressure—of the system can be changed without causing one of the phases to disappear. There is no degree of freedom, or, as it is sometimes expressed, the system is a non-variant system. The exact conditions for stability

[39] Morgan, "Physical Chemistry." New York, 1911, p. 119.

of such a system are a pressure of water vapor equal to 4 mm. of mercury and a temperature of .007° C. above 0° C.—the freezing point at atmospheric pressure.

Many applications of the phase rule to living matter have been made. We will cite but one. The globulins—typical proteins found in the blood of animals—are insoluble in distilled water, but are soluble in dilute solutions of the inorganic salts, such as sodium chloride. The globulin may exist in a system of water, sodium chloride and globulin, as globulin in solution or as precipitated globulin. The globulin is the only component existing in more than one phase under the conditions of the experiment.[39a] Addition of water to the system to such a degree that the concentration of the inorganic salts falls below a certain minimum leads to a precipitation of part of the globulin in solution. The removal of the mineral salts, keeping the volume of the solution constant, will also lead to precipitation wholly or in part, of the globulin in solution. But whether water be added or salt removed, the essential condition which undergoes changes is the concentration of the salt. Pressure does not enter in as one of the conditions of the system. And if the temperature of the system be raised above a certain point, depending upon the globulin present in the system, the globulin will be precipitated. In this system, the number of components is one (globulin) and the number of phases is also one, dissolved globulin. We have, therefore, a system of n components with n phases, and two of the conditions may vary, within certain limits, at the same time, viz., the concentration of the sodium chloride and the temperature. In the terminology of the quotation above, $P=1$, $C=1$ and $F=1+2-1$, or 2. This is also expressed by saying that the system is divariant. This system is of interest because of the fact that it also illustrates the phenomena of maximum points.

[39a] While globulin is not the only component entering into the system, we have restricted the discussion to the department of the globulin for reasons of space and simplicity.

Further details should be sought in the text-books of physical chemistry, and especially those by Bancroft and Findlay on the phase rule.[40]

IV. The Interpretation of the Regulatory Mechanisms in Terms of Chemical Equilibrium

But what evidence is there that the laws of mass action or of chemical equilibrium apply to living matter? Is there any evidence that the reactions occurring in the cell are "slow" reactions similar to those of the physicochemical laboratory? The answer to these questions is decidedly in the affirmative. Much evidence in favor of such a view was presented by Blackman. Hofmeister, Bredig and others regard the cell as a congeries of enzymes, each one, according to Hofmeister,[41] acting in its own compartment upon its own peculiar substrate.

1. *Applications of Van't Hoff's Law*

As further evidence of the nature of the reactions in living matter, we may cite the work of Shelford[42] on tiger beetles, in which the length of the combined quiescent periods of the pupal and the prepupal stages was increased from four or six weeks at a temperature of 28° to 30° C. to ten or twelve weeks at a temperature of 15° to 17° C. Riddle[43] found that the temperature coefficients for digestion in *Amia, Rana, Necturus* and the common turtle (*Emydoidea*) ranged from 0.93 in *Necturus* to 7.81 in the turtle. Rogers and Lewis[44] have recently shown that the temperature coefficient of the rate of contraction of the dorsal blood vessel of the earthworm is of the order of magnitude to be expected if the processes

[40] Bancroft, "The Phase Rule," Ithaca, New York; Findlay, "The Phase Rule and Its Applications," 3d ed., 1911, London and New York.

[41] Hofmeister, *loc. cit.*

[42] Shelford, *Linnean Society's Journal*, 1908, XXX, p. 176.

[43] Riddle, *American Journal of Physiology*, 1909, XXIV, pp. 447–458.

[44] Rogers and Lewis, *Biological Bulletin*, 1914, XXVII, p. 269. See also Lehenbauer, "Physiological Researches," 1914, I, pp. 247–288.

concerned are of the nature of slow chemical reactions. The application of Van't Hoff's law in these instances is sufficiently plain.

Considering the processes in living matter, from this point of view, we may gain some insight into the reason why so many of the factors or conditions entering into the reactions occurring in the body of a higher organism should be kept as nearly constant as possible.

2. *The General Conditions of the Reactions in the Cells*

In determining the velocity of a reaction, we may determine (1) what quantity of the reacting substances combine or react in unit time, the usual method of the laboratory, as has been shown above, or (2) we may determine what quantities of material must be added in unit time to keep the reaction going at a constant rate. Recalling now the nearly constant factors in the higher mammalian organism, the oxygen content, the temperature, and the hydrogen ion concentration all varying within relatively narrow limits, and the variations usually being in such a direction as to get more material to an active or working structure in unit time, we can see that there are certain very effective devices for maintaining a reaction at a constant speed, which are the counterparts of the apparatus employed in the chemical laboratory. But the mechanisms in the living organism are capable of regulating, with a great degree of exactness, more conditions than any artificial mechanisms so far devised in the laboratory can control.

In the evolution of the organism the development of the various regulating mechanisms which we have described has brought about a set of conditions which tend to keep the environment surrounding the cells relatively constant. The analogy beween the reactions in the cells and the slow reactions of the physical chemist becomes clear. The temperature of the body being constant, the reactions in the cells, dependent as they are, upon a constant supply of material, go on at a relatively constant rate, or at such a rate as is determined by the needs of

the organism, and a rate which is provided for by the changing distribution of the blood.

Not all the physico-chemical conditions of cell activity are as constant as those discussed in the second division of this paper. Nor does the experimental interference with certain body structures leading to known departures from the usual conditions always entail serious results. As an instance of this we may cite the experiments of Ogata,[45] who investigated the rate of absorption of protein when fed by the mouth as compared with its rate of absorption when introduced directly into the intestine through a fistula. Taking the nitrogen output in the urine as the expression of the rate of absorption, the nitrogen output rose much more rapidly after direct introduction of the meat into the intestine than it did when the meat was fed by mouth. Although the absorption of the food was apparently more rapid than usual, the capacity for adjustment on the part of the organism was not exceeded. We may mention in passing that one function of the stomach may be to act as a storehouse and provide for a more gradual absorption of food than would otherwise occur. In the terminology of this paper, there is a less sudden entrance of constituents tending toward a disturbance of the equilibrium when the stomach is present than when it is absent. If food is administered in small portions and in a finely divided state after complete removal of the stomach, life goes on as usual (Czerny). But one is hardly justified in saying that, because great and profound changes do not occur in the organism after extirpation of the stomach, the stomach has no important function.

A detailed consideration of the inconstant or variable conditions and of the manner and extent to which changes in the environment can influence all internal conditions, must be deferred for another communication. Enough has been said in these pages to show, in outline at least, the essential uniformity of some important internal con-

[45] Ogata, *Archiv für Anatomie und Physiologie*, 1883, p. 89.

ditions of the higher organism and to indicate their rôle, on the assumption that the internal mechanisms of the organism are physico-chemical mechanisms.

In the response of the respiratory mechanism to the increased concentration of carbon dioxide or to lack of oxygen in the blood, we have an instance of adaptation which is not at once seen to be an obviously automatic and inevitable result of the physico-chemical properties of the environment. A striking characteristic of the respiratory center is at once its sensitiveness to slight changes in the concentration of carbon dioxide and its tolerance to the accumulation of carbon dioxide in the blood. The respiratory cells react to an extremely slight increase of carbon dioxide which is insufficient to affect the other cells, and remain sensitive to this increase after the concentration has risen so high that the visible responses of certain other cells have ceased. The common excitability of the respiratory and other motor nerve cells to carbon dioxide may be supposed to result from the disturbance or change produced in a complex system by the accumulation of one end product of the reaction, and to this extent to be an automatic result of the physico-chemical constitution of the cell.

The question raised by Mathison[46] as to whether carbon dioxide is a stimulant for all nerve cells is of interest in this connection. Carbon dioxide is certainly a stimulant for the central nerve cells of the respiratory mechanism, but it is not necessarily a stimulant to the same degree for all nerve cells. It is probable that all living matter is more or less sensitive to the accumulation of carbon dioxide since it is one of the waste products of all destructive metabolism. The cell bodies of the respiratory neurones, by reason of the development of this common property of excitability to carbon dioxide, have become especially adapted to respond to slight variations in carbon dioxide. The adaptation undoubtedly depends upon a physico-chemical change in the respiratory neu-

[46] *Journal of Physiology*, 1910, XLI, p. 448.

rones. The persistence of the common property accounts for the asphyxial convulsions of the spinal animals and for the movements which are sometimes considered to be respiratory movements and commonly attributed to so-called respiratory centers. But whether we consider that the cells of the respiratory group have gradually acquired a lower threshold value for stimulation by carbon dioxide than the other cells of the nervous system, or that the cells of the respiratory group have simply retained the the common excitability of protoplasm in general to carbon dioxide, and the remaining cells have undergone modifications which have raised their threshold value, makes little difference from the theoretical point of view. In an environment which is, so far as one can determine, uniform, certain quantitative variations have occurred which have resulted in a differential sensitiveness to carbon dioxide or to lack of oxygen. The changes have not been qualitative, since asphyxial convulsions involving muscles innervated from other parts of the central nervous system, may be brought on by a reduction of the oxygen supply.

The usefulness of the lower threshold value for lack of oxygen in a particular group of cells is at once apparent. Oxygen generation of the blood and elimination of carbon dioxide proceed without attention, and without noticeable excitation of any other group of nerve cells. There is no disturbance of the precision of movement of any group of muscles aside from those actually engaged in the respiratory act, nor the slightest effect upon the neurones involved in mental processes, resulting from the decreased oxygen or increased carbon dioxide tension in the blood sufficient to provoke a respiratory response.

3. *Stimulation in Terms of Chemical Equilibrium*

This brings us to a consideration of the nature of stimulation in general. Lack of space precludes all but the briefest mention at this time. We may here simply indicate the consideration in terms of the laws of mass

action and of the phase rule. That changes in the speed of reaction depend upon the concentration of the reacting substances or of the end products of the reaction has been shown in the discussion of the laws of mass action. It is not difficult to see that the respiratory movements owe their origin largely to changes in concentration of carbon dioxide and oxygen, and, since these changes result in a slight change in the concentration of the hydrogen ions it is not difficult to imagine that the law of mass action may be involved in the stimulation of the respiratory cells in the medulla oblongata. We have given in the third section of this paper two illustrations of conditions coming under the operation of the phase rule. It is true that living matter undoubtedly comprises vastly more complex systems than those described, but that the general principles underlying the reactions are similar in most important respects to the systems employed in laboratory experiments is scarcely to be doubted. The withdrawal of water from a cell or nerve fiber by osmosis or drying, entailing a quantitative change in the amount of water in the cell, is followed by other changes in the cell which tend to bring about a reestablishment of conditions in accordance with the laws of chemical equilibrium. That a change of phase of some of the components occurs in the process is probable.

Such influences as drying, applications of heat or mechanical pressure, whether occurring in the laboratory or in nature, are known as stimuli, and the changes associated with their operation in the organism are responses to stimulation. As Jost[47] has pointed out, the formal conditions of existence may act as stimuli to organisms. Although we must admit that a wide gap still exists, it seems to us that the discussion of stimuli and the processes of stimulation in terms of the law of mass action and the phase rule will enable us to meet in some degree, however small, Haldane's objection[48] that no causal con-

[47] Jost, "Pflanzenphysiologie," zweite Aufl., p. 618, Jena, 1908.

[48] Haldane, *loc. cit.*, p. 37.

nection has been shown between stimulus and response. And we may hope that here as elsewhere in biology the limits to our knowledge of nature will gradually be broken down.

The accumulation of waste products in the blood or body fluids through increase of their concentration in these fluids, leads to modified activity of the excretory and other organs. We cite a few examples.

The kidney, in addition to the elimination of water, follows the law of mass action in other ways. The volume of urinary secretion, other things being equal, is proportional to the volume of blood flow through the kidneys in unit time. The greater volume of blood carries with it a greater volume of waste products in unit time, and hence a greater volume of secretion is the result to be expected if urinary secretion is a physico-chemical process following the general provision of the mass law.

The accumulation of waste products arising from the slow reactions in the cells gives rise to the phenomena of fatigue, and the general slowing down of the cell processes, just as the accumulation of the end products of any slow reversible reaction decreases the amount of chemical transformation in unit time, in accordance with the mass law.

Excess of carbon dioxide, a typical waste product, even of the activity in nerves,[49] decreases or abolishes the conductivity of a nerve fiber. A stimulus (geotropic) may be applied to a plant in an oxygen-free atmosphere, but the responses will not occur until the plant is moved to an atmosphere containing oxygen.[50]

But even waste products in a certain concentration may be necessary for the optimum conditions of activity of an organ. Baglioni[51] points out that the selachian heart maintains its activity better in a solution of the inorganic salts containing two per cent. urea—the normal

[49] Tashiro, *American Journal of Physiology*, 1913, xxxii, p. 107.

[50] Jost, ''Pflanzenphysiologie,'' zweite Aufl., p. 524, Jena, 1908.

[51] Baglioni, *Zeitschrift für allgemeine Physiologie*, 1906, VI, p. 71.

concentration of this substance in selachian blood—than in similar solutions without the urea.

V. General Considerations and Summary

The higher organisms have, therefore, developed a system of regulation by means of which internal conditions are kept relatively constant. This mechanism consists essentially of a physical means of distribution of material and heat—the circulatory organs and fluids—whose composition varies within narrow limits, a muscular, a glandular and a nervous mechanism for regulating the temperature, and a system of excretory organs for removing the waste products from the circulating fluids. Both chemical and nervous mechanisms of coordination are involved. The variations in the composition of the circulating fluids are such as will provide greater quantities of easily utilizable material at a time when it is needed. The internal secretions are important agents in maintaining the organism at a high pitch of efficiency through their influence upon the neuro-muscular apparatus and the general metabolism of all the tissues and organs. Regardless of the variations in external conditions, so long as these do not transcend the limits within which life is possible, and barring physical accidents or disease, the internal mechanisms keep it always fit, whether for work or rest, for battle or for play.

We have heard much about the survival of the fittest, and about the rôle of the strong jaw and powerful teeth and other physical characteristics in the struggle for existence. The doctrine of evolution, so far as its morphological side is concerned, may be regarded as fairly well founded. A little reflection, however, will show that the morphological aspect is only one phase of the problem. What profits it an animal to possess strong muscles and sharp teeth unless these muscles shall be at all times ready to contract quickly and surely? What if it become engaged in combat with an adversary and its muscles be

sluggish from cold? Or, supposing the temperature to be favorable, it be not able to control those muscles accurately and sink its teeth into the vital spot of the enemy? The answer is simple; another skeleton will soon lie bleaching. Somewhere or other evolution must have been concerned with the functional side. One protective mechanism has been suggested by the slow action of muscles in the cold and their more rapid action at higher temperature. The combat between a dog and a snake may be a fairly even one when the weather is warm, and very much in favor of the dog when the weather is cold. There is a strong presumption that the elaborate and complicated nervous vascular and glandular mechanisms, some or all of which are developed in birds and mammals, have some bearing on the general problem of evolution. It has rendered them far more independent of the environment than poikilothermal animals are. There is not so much necessity of hibernation during the winter, and a frosty morning is as good as any for hunting.

And if we consider that the changes of energy and material underlie all the other changes in the organism, regardless of the source from which they arise, it will be apparent that at least one part of the final discussion of evolution will be in terms of the changes of matter and energy within the organism.

The problems of the general processes of evolution—the adjustment of the animal to its environment or responses to changes in it, variation, adaptation, heredity and geographical distribution, and even the biochronic equation (De Vries) may all be approached from the point of view of the experimental physiologist. The consideration of these subjects will be taken up in subsequent papers.

CORRELATION BETWEEN EGG-LAYING ACTIVITY AND YELLOW PIGMENT IN THE DOMESTIC FOWL[1]

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So far as the presence of visible yellow pigment is concerned, there are two groups of domestic fowls. In the first group, represented by the Orpington breed, yellow is constantly absent from legs, beak and body fat. In the second group, represented by the Leghorns and the so-called American breeds, such as the Plymouth Rocks, Wyandottes and Rhode Island Reds, yellow, in the form of yellow fat,[2] is present in varying amounts in the parts mentioned. In this latter group, individual birds may undergo considerable change in the amount of the yellow pigment visible. The standard of the show-room, however, demands yellow in the legs and beak in these breeds and, in consequence, birds that have become pale in these parts are liable to be scored down by the professional poultry judge. The paling or yellowing of the legs in the breeds mentioned has been attributed by poultrymen to various environmental factors. Thus, good health and vitality, abundance of range and exercise, proper food such as meat, corn, gluten meal and "green food" are said to increase the amount of yellow pigment, while poor health, moulting, confinement with insufficient exercise, running on sandy soil and in mud, as well as climate and the mere aging of the bird, are held to be responsible for the paling of the legs in these varieties.

Of recent years, some individual poultrymen have claimed that paling of the legs is due to heavy laying. This view has been maintained by J. E. Rice.[3] Mr. Tom Barron,[4] one of the most successful of the English poultrymen, in an address before the Connecticut Poultry

1 Paper presented in brief before the American Society of Naturalists, Philadelphia, December 31, 1914.

2 Barrows, H R., "Histological Basis of Shank Colors in Domestic Fowl," Bull. 232, Maine Agric. Exper. Station, 1914.

3 Circular 54, N. Y. State Dept. of Agriculture, 1912.

4 *Connecticut Farmer*, September 12, 1914.

Convention, July, 1914, described his use of the color of the legs in selecting high egg-producers. Moreover, the Maine Experiment Station, in a circular[5] which has come to our notice since the data in the present paper were obtained, advocates a similar use of the leg color in selecting hens for breeding.

The requirements of the "standard of perfection," which controls judging in the show room, as well as the common practice of poultry breeders, are opposed to a belief in any connection between laying and leg color. Woods,[6] under the title, "Has Leg Color Value Indicating Layers?", discusses the subject and concludes:

> Personally we believe that, as a practical guide in the selection of heavy layers or birds from which to breed heavy layers, the leg color, of itself, has no real value.

He further supports this conclusion by quotations from answers received from several prominent breeders to whom he had addressed a questionnaire on the subject.

So far as the writers are aware, no published data are available which show in how far the leg color may be of any value in selecting the laying hen, and such suggestions as have been made in this connection have confined themselves to a consideration of the legs alone. The results tabulated in the present paper show conclusively, it is believed, that a close connection does in fact exist between the yellow pigmentation in a hen and her previous egg-laying activity. They indicate further that the color of the beak is at least as distinctive as that of the legs heretofore alone considered in this connection, and that, in the Leghorns, the color of the ear-lobes is perhaps a better criterion of laying activity than either legs or beak and is more readily recorded.

The hens investigated were in the egg-laying contest located at Storrs, Conn. Pullets enter the contest November 1 and remain for one year. They are housed in pens of 10 birds each, are fed the same ration and so far as

[5] Circular 499, Maine Agric. Exper. Station. This is listed as an abstract of Bulletin 232.

[6] Woods, P. T., *Amer. Poultry Jour.*, p. 35, January, 1915.

possible are handled exactly alike.[7] The influence of different environmental factors, therefore, can be largely neglected.

A preliminary test was made the middle of last September by taking from each of a number of different pens a pair of birds representing the extremes of yellow pigmentation and comparing their egg records. This test indicated that the extremely pale birds were laying and the extremely yellow ones were not. It indicated also that the ear-lobes were much more easily graded as to color and in addition were apparently more indicative of egg-laying activity than the beaks and legs. The ear-lobes of the American breeds are red like the comb and wattles and do not show yellow pigment. The ear-lobes of certain other breeds, like the Blue Andalusians, are white but apparently remain without any appearance of yellow ever taking place. The Leghorns, including Browns, Blacks, Buffs and Whites, show marked changes in the amount of yellow in their ear-lobes. White Leghorns, of which there were over 300 in the contest, were accordingly chosen for closer study.

Ear-lobe Color in White Leghorns.—Color can be conveniently measured quantitatively by means of the Milton Bradley color top, which, when spinning, acts as a color mixer. In matching ear-lobes, only yellow and white sectors have been used. The matching is not perfect, especially in the lower grades, since a certain amount of bluish tinge is often present. The amount of yellow, however, has probably been more accurately measured than if the other color components were considered. By the method used, it appears possible under proper illumination for one to repeat readings with a change of seldom more than 5 per cent. yellow above or below the mean observation.

Top readings were taken of the White Leghorns listed

[7] Four pens of White Leghorns and four of White Rocks, belonging to the Experiment Station, had sour milk substituted for different ingredients of the normal ration, but, since they showed no apparent differences in color that could be attributed to the change in the feed, they were included in the tabulations.

TABLE I

AVERAGE EGG RECORDS FOR DIFFERENT AMOUNTS OF YELLOW IN EAR-LOBES OF 312 WHITE LEGHORNS

Per Cent. Yellow	No. of Hens	July	Aug.	Sept.	Oct	I Sept.	II Sept.	I Oct.	II Oct.	Year
5–10	7	23.1	21.3	19.7	15.3	9.9	9.9	9.29	6.00	197.1
11–15	36	21.8	22.1	18.2	14.2	9.4	8.8	8.14	6.03	187.9
16–20	40	22.2	20.7	16.9	11.7	8.8	8.2	7.50	4.17	184.3
21–25	16	19.8	21.4	16.4	8.1	8.1	8.3	5.56	2.50	164.3
26–30	20	19.5	18.9	10.3	3.2	5.5	4.8	2.75	0.45	148.5
31–35	31	18.0	17.7	5.5	0.5	3.5	2.1	0.45	0.00	139.1
36–40	33	19.7	17.3	6.1	0.2	4.2	1.9	0.15	0.00	139.6
41–45	41	18.2	16.2	4.9	0.2	3.4	1.6	0.22	0.00	134.2
46–50	39	18.0	15.6	4.0	0.2	2.6	1.4	0.18	0.05	138.2
51–55	30	18.4	16.1	3.6	0.1	2.9	0.7	0.00	0.07	137.8
56–60	13	14.8	10.7	2.4	0.0	2.2	0.2	0.00	0.00	124.7
61–65	4	14.5	8.8	1.3	0.3	0.3	1.0	0.25	0.00	100.8
66–70	1	3.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00	70.0
71–75	1	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00	83.0

TABLE II

PERCENTAGE OF HENS LAYING AND AVERAGE NUMBER OF DAYS SINCE LAYING FOR DIFFERENT AMOUNTS OF YELLOW IN EAR-LOBES

White Leghorns: total number of records, 932; total number of birds, 317

Per Cent. Yellow	5–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70	71–75
No. records	41	125	80	67	62	92	94	94	108	84	44	28	9	4
Av. days since laying	0.4	1.6	7.3	17.1	26.2	37.9	41.5	44.0	45.1	51.3	55.9	61.4	50.3	71.0
No. records = laying	36	98	44	17	3	0	1	0	2	0	0	0	0	0
Per cent. records = laying	87.8	78.4	55.0	25.4	04.8	0	01.0	0	01.9	0	0	0	0	0

in Tables I and II at three different periods. The first recording took from October 7 to October 14. the second from October 19 to 21 and the last was completed in one day, October 28. The top records were all made by the same one of us (B), except for 197 records on October 28. The men who took these records had already acquired familiarity with the method, and while their readings are not absolutely comparable with the others, they probably are sufficiently so to be included in Table II. The three top readings were taken on separate sheets and the egg records were investigated after the readings were all taken and the birds had left the contest. Personal bias that might have influenced the readings was thereby avoided.

Table I shows the percentage of yellow in the ear-lobes

of 312 birds according to the records of October 19–21, together with monthly and yearly egg records for the different color groups. The months of October and September are each divided into halves. It will be seen that in general as the percentage of yellow increases, the egg production falls off, and that the correlation is most marked during the periods nearest the time when the records were taken. A distinct though slight correlation seems to show as far back even as July and is strikingly evident in the yearly averages. For months before September and October, the correlation with color is probably an indirect one. It is generally only the best birds—those that make the large yearly records—that are laying in October. Therefore, any method that selects the laying birds at this season will select, at the same time, the birds laying above average throughout the year and consequently give high yearly totals. It will be observed that 30 per cent. seems to be a critical amount of yellow. Above this amount comes the sudden drop in egg production for the months of September and October and also above 30 per cent. yellow the yearly totals fall to between 130 and 140, with but slight change thereafter.

In Table II, the records at the three different readings have been used. A bird laying on the day of record or on a later day within the month is considered to be laying and credited with a zero. If she laid on the day before the record but not later, she is credited with one "day since laying," and in a similar way a longer period of inactivity in laying is indicated by a larger number of days since laying. With the exception of a few cases where this was not possible, three records were taken of each bird. Since October is the season of decreasing egg production, the majority of the birds increased their quantum of yellow and consequently most birds are listed in more than a single color grade. Beginning with the 41 records in the 5–10 per cent. color grade which show an average of only 0.4 day since laying, the number of days increases consistently with the amount of yellow in the ear-lobes, the irregularity at 70 per cent. being probably due to the

smallness of the numbers in this group. The percentage of records that indicate actual laying drops rapidly from 87.8 per cent. for 5–10 per cent. yellow to zero for grades of yellow above 30 per cent.[8] The table shows that it is practically certain that a bird with an ear-lobe showing more than 30 per cent. yellow at the time of the records, is not in a laying condition.

TABLE III

AVERAGE EGG RECORDS FOR DIFFERENT GRADES OF YELLOW IN BEAKS AND LEGS OF 256 WHITE LEGHORNS

(P, M and Y are abbreviations for Pale, Medium and Yellow)

No. Birds	Beak	Legs	July	Aug.	Sept.	Oct.	I Sept.	II Sept.	I Oct.	II Oct.	Year
51	P	P	22.0	20.9	18.6	14.3	9.6	9.0	8.0	6.3	186.4
17	M	M	18.5	17.8	11.4	4.8	6.5	4.9	3.4	1.4	146.4
97	Y	Y	16.6	14.2	2.9	0.4	2.2	0.7	0.3	0.1	129.3
53	P	P 51, M 2, Y 0	22.1	21.0	18.3	14.0	9.4	8.8	7.9	6.1	185.3
43	M	P 25, M17, Y 1	20.1	20.6	13.7	5.7	7.4	6.3	4.2	1.5	164.6
160	Y	P 14, M49, Y 97	17.7	16.1	4.8	0.8	3.2	1.7	0.6	0.2	135.0
90	P 51, M25, Y 14	P	21.6	21.3	17.0	10.4	8.9	8.2	6.3	4.1	179.9
68	P 2, M17, Y 49	M	19.2	18.3	7.7	2.1	4.7	3.0	1.5	0.6	142.0
98	P 0, M 1, Y 97	Y	16.6	14.3	2.9	0.4	2.1	0.7	0.3	0.1	129.2
256	Averages of totals		19.0	17.8	9.1	4.3	5.2	3.9	2.7	1.6	150.4

Beak and Leg Color.—The beak and legs are more difficult to grade quantitatively than the ear-lobes. The color is less uniform in its distribution and has more of an orange hue, requiring the manipulation of at least one

[8] The three cases of laying, among the 557 records in the grades above 30 per cent. yellow were for sporadic layers. The one in the 40 per cent. group laid October 18, but at no other time in October or September. This case may perhaps be an error in the egg record. One of the two in the 50 per cent. grade laid during October only upon the 2d, 4th and 25th, though she laid 18 eggs in September; the other laid during October only on the 16th and 19th and had no eggs to her credit in the second half of September.

extra color disk in taking the records. A rough grouping by inspection into the three grades, *pale, medium* and *yellow,* however, gives a striking corroboration of the results obtained by the more accurate records on the ear-lobes and is applicable to breeds in which ear-lobe yellow is not present. The grading was always done by the same one of us (W.) who has had some familiarity in handling poultry. Probably no two observers would entirely agree in recording the colors but the difficulty comes in delimiting the grade *medium,* and not in deciding between the extremes, *pale* and *yellow.* The color records were taken on October 31 and November 1 to 4, as the birds were being packed for shipment and their egg records were looked up for tabulation after they had left the contest.

Table III corresponds to Table I. In the first three rows are listed the birds that agree in beak and leg color. In the second three rows the birds are grouped according to their beak colors without regard to their leg colors, while in the last three rows they are grouped according to leg color alone.

Table IV corresponds to Table II. Since egg records for these birds stopped on October 31, a bird laying on October 29 is counted among the layers even if she failed to lay on the 31st—the day she left the contest.

It will be noted from Table III that, in the Leghorns at least, where the numbers are large enough to make comparisons significant, the beaks, considered alone, seem to form a slightly better criterion for picking out the hens with high records, while the legs alone are better in selecting the poorest layers. In the great majority of cases in all the breeds considered, if the beak and the legs fail to agree in color it is the beak that is listed the yellower. In October the hens are falling off in laying and in consequence increasing in yellow pigment. Apparently the ear-lobes and beak are more quickly responsive to this change. In only 97 out of 160 Leghorns for which the beak was listed as yellow had the legs reached a similar grading in color.

Of the 51 White Leghorns listed in Table III as pale in

both legs and beak, 31 had ear-lobe records of 20 per cent. or less yellow on October 28. These averaged a yearly total of 191.9 eggs. The 40 birds of those in Table III that on this date had 20 per cent. or less yellow in ear-lobes, irrespective of the color of other parts, averaged a yearly total of 189.4 eggs. It appears therefore that hens with a higher yearly average may be obtained by selecting those that are pale in all parts—ear-lobes and beak as well as in legs—than if only one of these parts is considered.

TABLE IV

PERCENTAGE OF BIRDS LAYING, AVERAGE NUMBER OF DAYS SINCE LAYING AND YEARLY TOTALS FOR DIFFERENT COLOR GRADES OF BEAKS AND LEGS

(P, M and Y are abbreviations for Pale, Medium and Yellow; the color of beak is written first, followed by color of legs)

White Leghorns (256 birds with yearly average of 150.4 eggs)

	P.P.	M.M.	Y.Y.	P.M.	P.Y.	M.P.	M.Y.	Y.P.	Y.M.
No. birds	51	17	97	2	0	25	1	14	49
Av. days since laying	6.6	30.4	57.8	30.5	—	20.8	64.0	28.6	45.9
No. birds laying	32	2	1	0	—	3	0	1	1
Per cent. birds laying	62.8	11.8	1.0	0.0	—	12.0	0.0	7.2	2.0
Yearly averages	186.4	146.4	129.3	150.5	—	178.7	122.0	158.4	139.9

White, Buff and Columbian Wyandottes (79 birds with yearly average of 144.8 eggs)

	P.P.	M.M.	Y.Y.	P.M.	P.Y.	M.P.	M.Y.	Y.P.	Y.M.
No. birds	28	13	24	1	0	4	0	0	9
Av. days since laying	6.5	17.5	48.9	0	—	7	—	—	28.7
No. birds laying	16	5	0	1	—	2	—	—	1
Per cent. birds laying	57.2	38.5	0.0	100.0	—	50.0	—	—	11.1
Yearly averages	178.3	130.7	108.4	194.0	—	161.5	—	—	145.6

Barred, White and Buff Plymouth Rocks (82 birds with yearly average of 142.4 eggs)

	P.P.	M.M.	Y.Y.	P.M.	P.Y.	M.P.	M.Y.	Y.P.	Y.M.
No. birds	27	15	22	3	1	5	2	3	4
Av. days since laying	11.3	25.9	51.6	1.0	0.0	33.8	6.0	29.3	31.5
No. birds laying	15	3	0	2	1	0	1	1	3
Per cent. birds laying	55.5	20.0	0.0	66.6	100.0	0.0	50.0	33.3	75.0
Yearly averages	153.7	149.7	123.0	163.7	204.0	138.8	117.5	130.7	140.0

Rhode Island Reds (114 birds with yearly average of 128.8 eggs)

	P.P.	M.M.	Y.Y.	P.M.	P.Y.	M.P.	M.Y.	Y.P.	Y.M.
No. birds	26	15	56	0	0	3	1	4	9
Av. days since laying	11.3	15.8	50.4	—	—	19.0	38.0	18.3	41.2
No. birds laying	14	7	2	—	—	0	0	1	1
Per cent. birds laying	53.9	46.7	3.6	—	—	0.0	0.0	2.5	1.1
Yearly averages	146.3	142.5	109.8	—	—	159.0	178.0	162.0	139.2

The method of grading beak and leg color may appear crude, but that it is capable of giving valuable evidence of previous laying activity is further shown by data kindly turned over to us by Professor C. A. Wheeler. On October 26, 1912, under his direction a series of measurements of 132 White Leghorns from the contest was taken by Mr. R. E. Jones. Among other records, the ear-lobes were graded as white, cream or yellow and the legs as pale or yellow, but no connection was worked out between the color and the egg records. These 132 birds we find to have a yearly average of 155.1 eggs. The 34 birds with pale legs averaged 188.9 eggs; the 98 with yellow legs, 143.5 eggs. The 33 birds with white lobes averaged 190.1, while the 99 with cream or yellow lobes averaged 143.5. The 21 birds that had both white ear-lobes and pale legs averaged exactly 200 eggs.

The data presented in the foregoing pages indicate a connection between the amount of yellow pigment showing in a hen and her previous laying activity. The most natural assumption is that laying removes yellow pigment with the yolks more rapidly than it can be replaced by the normal metabolism, and in consequence the ear-lobes, the beak and the legs become pale by this subtraction of pigment.

Environmental factors, other than laying, may be of more or less influence on yellow pigmentation. In fact, birds obviously sick have been observed to be pale although not in a laying condition. In the material investigated, however, variation in the laying activity seems to be the prime cause of the changes in yellow pigmentation in the domestic fowl.

The data of the present paper have been summarized in a preliminary report in *Science,* March 19, 1915. Photographs showing differences in yellow pigmentation in fowls are given in an article in the *Journal of Heredity,* April, 1915.

The change in yellow pigmentation is being further studied by a twice weekly top record of a flock of birds throughout the year.

SOME RECENT STUDIES ON FOSSIL AMPHIBIA

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The anatomy and relationships of the earliest air-breathing vertebrates have interested students of fossil animals so greatly since Georg Jaeger described the first Labyrinthodont in 1828, that the result to-day is a bibliographic list of over 600 titles, varying in importance from the magnificent work of Fritsch ("Fauna der Gaskohle") issued in four folio volumes with scores of lithographic plates, to short notices of a few lines. Many of the memoirs are handsomely illustrated and beautifully printed. The material so far described has been extremely fragmentary and the greater number of the contributions issued have been dedicated to the description of species based on incomplete material. The fauna was exceedingly diverse like the plesiosaurs of a later period, and new discoveries tend to confuse rather than to unify our ideas of amphibian morphology. The few papers reviewed below form no exception to the statement made above. Many new and importants facts are brought forth in the contributions made during the past few months and these are well worthy of consideration. Attention in these reviews will be paid especially to new facts of structural importance.

Broili (1) in a short paper has added to our knowledge of the Permian fauna of Texas by the description of two new species of Amphibia based on incomplete skulls. One of the species is very small, the skull measuring scarcely half an inch in length. The same writer (2) in a more extensive paper has given a popular review of the chief work done during the past ten years on the early air-breathing vertebrates and has listed the important papers from which he has used illustrations to elucidate his remarks. This paper should be consulted by any one who wishes a convenient and accurate survey of the earliest land vertebrates. Doctor Broili refers to *Micrerpeton*, the first branchiosaur known from the western hemi-

sphere, as a microsaur. The distinction between these two groups is clear, the former undoubtedly being ancestral to the modern Caudata and the latter having reptilian affinities. Likewise the author refers to *Lysorophus*[1] as a reptile, while the majority of paleontologists regard the form as Amphibian; Williston[1] even going so far as to locate it in the suborder Ichthyoidea of the Caudata. In conclusion Doctor Broili says:

> Im übrigen haben wir im Laufe der letzten 10 Jahre über die ältesten Tetrapoden so viel neues und wichtiges kennen gelernt, wie wohl in relativ keinem anderen Zweige der Wirbeltierpaläontologie. . . .

Broom (3) has given the results of his studies on Permian vertebrates in the American Museum. His reason for again describing and studying this much described and much studied material is that structural characters are difficult to determine in these forms on account of the very closely adherent matrix which has in many cases obscured all sutures in the skull. His discussion is accompanied by restorations of the skulls of the chief Permian genera, indicating most of the sutures, something which Cope was unable to do. He discusses some elements in the mandible not previously observed among Amphibia and suggests homologies between them and elements of the reptilian mandible. Unfortunately, Broom has paid no attention to the occurrence of lateral line canals on the skulls of these forms. It is highly important that this system of sense organs be distinctly understood. In view of Herrick's studies[2] on this structure in the catfish it is certain that this system of sensory organs has a distinct influence on the location of the peripheral osseous elements of the skull and mandible. I do not recall that Herrick's result have been noted by any paleontologist, but they should be taken into consideration. Broom says in regard to *Eryops*, the large Permian stegocephalian:

> . . . Every detail of the cranial structure can be clearly made out.

He criticizes Huene's (1913 *b*) work on the brain-case, however, and makes no statement concerning the lateral

[1] *Biol. Bull.*, Vol. XV, No. 5, p. 229, 1908.

[2] *Journ. Comp. Neurol.*, Vol. 11, p. 224, 1901.

line organs which were imperfectly studied some years ago[3] by the reviewer; so it is yet too soon to say that every detail of structure is known. The palate is very completely known and is figured by Broom. He has figured also very imperfectly, but for the first time, sections through the ear and brain-case showing the probable size of the dural cavity. He says that the portion of the paroccipital which lodges the labyrinth was cartilaginous, but does not give his reasons for this statement. In view of the almost perfect preservation of the semicircular canals in fishes, cotylosaurs and pterodactyls we should expect a favorably preserved specimen of an amphibian to show this structure also. He describes a pit in the basisphenoid for the reception of the hypophysis. He also figures for the first time the complete osteology of the mandible of *Eryops*. The author likewise describes and briefly figures two new species of stegocephalians. The same author (4) has given considerable attention to the study of the osteology of the mandible in *Trimeorohachis*, the discussion being very similar to that given in the above paper. The discussion has especially in view the problem of the derivation of the Amphibia from the Crossopterygia, and he figures the mandible, shoulder girdle and pectoral fin of *Sauripteris taylori* on account:

> . . . of (the) extreme interest from having the pectoral fin more closely resembling the tetrapod limb than in any other known form.

Case (5) reviewed before the American Paleontological Society the recent trend of studies on the air-breathing vertebrates of the Paleozoic. He states there are two general conclusions which have been reached by students of these early vertebrates. First, Baur initiated the idea of the crossopterygian ancestry of the Amphibia, and later workers have so far confirmed his suggestion as to make it extremely probably that the land vertebrates arose from these fishes. The intermediate stages are unknown. The second conclusion is

> that the primitive reptiles—the Cotylosauria—were derived directly from the Stegocephalia. . . .

So we are thus in possession of partial proof at least of

[3] *Journ. Morphol.*, 1908, Vol. XIX, p. 511.

the origin of reptiles from fishes through the Amphibia.

We owe to Doctor Fraas of Stuttgart many important contributions to the knowledge of the early air-breathing vertebrates and he has recently (6) issued another memoir on the labyrinthodonts of the Trias, the first study of these animals since the appearance of his large memoir in 1889.[4] The present contribution is devoted to discussions of new species and new facts concerning previously described species. The *Plagiosternum granulosum* is found to be the most peculiar labyrinthodont yet described, in that it is extremely frog-like in appearance, especially in the huge size of the orbits and the expanded occiput. It is interesting, furthermore, in the apparent absence or indistinct preservation of the lateral line canals. The photograph (Plate XVI, Fig. 1) of the dorsum shows portions of the supra- and infraorbital canals. The remainder of the cephalic system of sense organs was probably contained in pits, which, in the fossilized skull, are not to be distinguished from the ornamental scrobiculations of the membrane bones of the face. The auditory meatus is on the posterior edge of the skull and is quite large for the size of the skull. Doctor Fraas has given in a drawing (Plate XVI, Fig. 3) the complete osteology of the occiput of this unusual labyrinthodont. The remainder of the memoir is devoted to a discussion of new or disputed points in the osteology of various genera and species of Triassic labyrinthodonts.

Gregory (7) has reviewed the studies which have thrown light on the crossopterygian ancestry of the Amphibia, dealing especially with Watson's (11) recent paper on the Larger Coal Measures Amphibia, and giving a list of thirteen contributions which deal directly with this derivation of the Amphibia.

Huene (8) has again described the mandible of the peculiar Permian genus *Diplocaulus* although it has been many times studied, described and figured. He states, in his introductory paragraph:

> Gattungen, wie Diceratosaurus, Eoserpeton, Stegops, Amphibamus, vieleicht auch Tuditanus zeigen Verwandtschaft mit Diplocaulus.

[4] "Paleontographica," Bd. XXXVI.

Just what the basis of this relationship is he does not state. The reviewer[5] has previously stated that these above-mentioned microsaurian genera exhibit *no* structural features which would ally them, except remotely, with *Diplocaulus*. This Permian genus has no relatives among the Coal Measures Microsauria, the reasons for this statement being given in the above-mentioned essay[5] and need not be repeated here. The material on which von Huene bases his paper was collected in Baylor County, Texas, and formed a part of a collection purchased by Doctor von Huene from Charles Sternberg at Lawrence, Kansas. The same writer (9) has again studied the Permian *Lysorophus*, which is regarded by Williston as closely akin to the salamanders.[6] Huene bases his discussion on twenty-four skulls in the collection of the University of Tübingen. He describes and figures some minute limb bones, thus partially confirming Miss Finney's results.[7] He agrees with Williston that *Lysorophus* is related to the Urodeles though suggesting:

> Mit den Temnospondylia hat der permische Urodele Lysorophus noch grössere Ähnlichkeit als die jetzigen Urodelen. Sie liegen in der Schädelbasis und der grosseren Anzahl der hinteren Schädeldeckknochen.

The same author (10) gives the results of his studies of Permian vertebrates at the American Museum. The paper is illustrated by sketches of various skulls and parts of skulls made by the author and showing his interpretation of the elements composing the cranium of American Permian amphibians and reptiles. He describes and figures a *stapes* in a skull of *Eryops* and gives the results of his study of the brain-case of this genus. The *stapes* has a length of 4 cm. and in shape is not unlike a human clavicle. His studies of *Lysorophus, Gymnarthrus, Diplocaulus* and other genera confirm the results of previous students of these forms. He concludes his paper with a discussion of morphological results, and appends a bibliography of twenty papers.

[5] *Journ. Morphol.*, Vol. 23, p. 31, 1912.
[6] *Biol. Bull.*, XV, 1908, p. 229.
[7] *Journ. Morphol.*, 23, p. 664, 1912.

Watson (11) has restudied the skulls of some of the European Carboniferous labyrinthodonts, *Loxomma, Pteroplax,* and *Anthracosaurus,* and compared them with the Coal Measures fish, *Megalichthys.* His results have already been reviewed by Gregory (7), so that it will only be necessary here to say that these genera approach the crossopterygian type of structure in various features. The same author (12) has redescribed an interesting microsaur in which he is able to give a very complete account of the structure of the dorsal and ventral surfaces of the skull and pectoral girdle. He compares the newly reconstructed microsaurian with *Diplocaulus* and *Ceraterpeton.* It is very important that these little-known species from Europe be restudied and redescribed, so that former observations may be corrected, corroborated and extended. The status of

> The classification of the smaller stegocephalian Amphibia, so abundant in the Coal Measures and Permian Rocks of Europe and North America, is in such confusion, to which some recent work has added, that it is at present only possible to proceed by reference to individual specimens which have been well described.

The reviewer finds himself in hearty accord with these statements, although he must plead guilty of having thrown some confusion into the classification of these animals in the hope that thereby order might ensue.

Doctor Williston (14) has determined the complete osteology of the mandible in the early reptiles and amphibians, working especially with the material from the Permian of America. He says:

> In the structure of the mandible the amphibians are remarkably intermediate between the early reptiles and the contemporary crossopterygian fishes, differing from the latter chiefly in the reduced number of coronoids, and from the former chiefly in the possession of two additional coronoids and a splenial.

These results are corroborated by the studies of Doctor Broom on similar material, so that any doubts as to the real structure of the stegocephalian mandible are placed at rest by the results arrived at by these separate investigations.

The mandible of the primitive amphibians differs chiefly from that of the early reptiles in the division of the coronoid into three elements, or possibly four, and in the division of the splenial into two.

Wiman (15) within the past three years has become much interested in the amphibian fauna of the Trias of Spitzbergen. In the present paper he reviews the work which has been done on the structures of the occiput of seven genera of Permian and Triassic stegocephalians, figuring the anatomy of this region of a new labyrinthodont from Spitzbergen. He describes this new genus in a later contribution. In this latter paper (16) Wiman discusses the occurrence of amphibian remains in the deposits of Spitzbergen, accompanying his remarks by photographs of the bone-bearing horizons. His paper deals largely with new forms from Spitzbergen, which are illustrated in four text figures and nine photographic plates. One is at once struck, in the examination of Wiman's plates, by the clearness of preservation of the cephalic lateral line canals. The author refers to these structures as "Schleimkanäle" and gives a very careful description of their occurrence; the only writer of recent date who has done so. The term *Lyrocephalus euri* is proposed for the new genus and species.

Der Gattungsname bezieht sich auf die ausserordentlich kräftig entwickelten Schleimkanäle des Kopfes. . . .

He refers to the various canals as "Tremalkanäle," "Nasofrontalkanäle," "Temporalkanal" and "Maxillarkanal," but makes no attempt to homologize them on the basis of the work of Allis[8] (1889) and the reviewer[8] (1908). The lateral line canals are so unusually well preserved in *Lyrocephalus* that it is thought worth while to give an outline figure in another place of their occurrence and to homologize them on the basis of previous work. The *columella auris* is described and figured (Plate II, Figs. 4–5) in this species. It is unusually large. Other new forms are described from these interesting deposits, many of the specimens showing much of interest in a structural way. The material described is chiefly cranial, although a few thoracic plates (interclavicles), of the typical laby-

[8] *Journ. Morphol.*, II, 1889, p. 463; 1908, p. 511.

rinthodont form, are described and figured. Doctor Wiman is to be congratulated on his contributions to our knowledge of these early vertebrates. His future papers will be looked for with much interest.

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SHORTER ARTICLES AND DISCUSSION

THE RESEMBLANCE OF YOUNG TWINS IN HANDWRITING

By each of 144 children 7 to 15 years old, forming 72 twin pairs, the first name (and usually also a word or so like "years old" or March or grade) was written. These were pasted on cards identified by chance numbering. Twelve men and women of good general education, but of no special experience in identifying handwritings, were shown the 72 specimens belonging to 72 first members of twin pairs and asked to match each by the specimen of the remaining 72 which most resembled it.

There was thus one chance in 72 of a correct match by chance, or 12 chances for all judges combined. There were in fact 6, 4, 8, 4, 6, 6, 7, 1, 3, 6, 3 and 4 correct pairings made by the twelve judges, respectively, or 58 in all.

It would be possible by the same method to derive a scale for unintentional resemblance in specimens of handwriting as shown roughly below. Such a scale might indirectly be of use in the study of questioned documents, since the resemblance of one specimen of an individual's writing to another specimen by himself may be regarded as the limiting case of the unintentional resemblance found amongst different individuals. A scale for resemblances produced intentionally would presumably form a series in which the resemblances would, upon analysis, be found characteristically different from the unintentional or natural resemblances. The genuineness of a questioned specimen of writing might thus be determined in part by measuring its resemblance to the unquestioned specimen in the different elements characteristic of the two scales. Resemblances of certain sorts might thus be used as actual evidence of forgery, and differences of certain sorts as evidences of genuineness, more systematically and objectively than is now the case.

Specimens 145 and 147 have a curious interest as a result of possessing nearly as close resemblance between two "natural" writings by two different persons as is likely ever to be found. It is probable that if the individuals in question had each written

Fig. 1. Rough Scale of Resemblances in Handwriting. 145 and 147 were regarded as matches by 11 of the 12 judges; 19 and 65 were so regarded by 5 judges; 38 and 90, by 2 judges; 4 and 96 by none.

a hundred natural specimens of the same two words, and if judges of the training of tellers in banks had been given the task of separating the two hundred specimens into the hundred by individual *A* and the hundred by individual *B*, the percentage of failures would have been considerable. These specimens, that is, may illustrate the possibility of successful forgery without artifice.

In general, of course, the experiment shows how very, very rare the case of substantially perfect resemblance of two natural signatures by different individuals will be. One case amongst 72 pairs of twins probably signifies less than one case in a thousand amongst the general population of as close resemblance as 145

and 147. Twins are probably distinguishable by their handwriting oftener than by their physical appearance; for I am confident that the bodies of at least five and probably more of these 72 twins would have been as hard to tell apart from a minute's visual inspection as specimens 145 and 147. Of people in general this would probably not hold true. but the distinguishing value of a specimen of natural writing is very high even for them.

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ALLELOMORPHS AND MICE

IN the February number of this journal, C. C. Little points out that Cuénot (1903) recognized certain factors in mice as allelomorphic,[1] and that in my paper of 1914 I not only failed to mention that Cuénot had treated these factors in this way, but that I claimed to have brought forward for the first time a demonstration of the allelomorphism in question. In fact, I did overlook or had forgotten that Cuénot interpreted these types in this way; and curiously enough, my work was undertaken because Little on the alleged results of some of his own earlier experiments denied that the factors for yellow and gray are completely linked, despite Cuénot's evidence, then published, which Little now says established from the ratios obtained that the factors in question are allelomorphic.[2] Little wrote as late as 1913:

> "Yellow" in mice is no more allelomorphic to gray than is gray allelomorphic to black.

If this is the conclusion at which he arrived after his elaborate series of experiments and after Cuénot's work had been done, the need of further work would seem to be obvious.

The failure of several of us to fully appreciate the significance of Cuénot's statements and evidence in regard to allelomorphism may in part be due to the fact that in his second paper Cuénot had used the symbols G (gray) and N (black) as allelomorphs, and had besides used the symbols A (albino) and G (gray) as allelomorphs without, however, intending to mean that there was here a set of

[1] Note 1903, *Archiv. Zool. Exp. et Gen.* (4), I.

[2] The numerical results are the same for complete linkage and for multiple allelomorphs. The evidence that would disprove the one would also disprove the other.

three allelomorphs, but using G in each of the two cases to represent a different factor of the gray mouse.[3] This method is in itself quite legitimate, but as a result when Cuénot later spoke of the factor for gray, gray white belly, yellow, and black, all as allelomorphic, some of us, it appears, failed to appreciate that in doing so Cuénot was treating this set of terms in an entirely different way from the way in which he treated the other cases, where he had represented factors as allelomorphic to each other. In the second place, the full significance of multiple allelomorphs in mice was not, I think, fully appreciated until its relation to complete linkage became apparent, and in fact even now this relation is not sufficiently understood by many geneticists themselves. Even, however, had I taken fully into account all that Cuénot had done, the somewhat extensive experiments that I undertook in order to *prove* that the factors in question are allelomorphic would have seemed to me necessary, as they still do, to establish that this series of factors bears this relation to each other. Let us examine, therefore, the evidence, which, according to Mr. Little, rendered my experiments a work of supererogation.

1. Little says:

> As early as 1903 Cuénot recognized that albinos, potentially yellows, when crossed with black gave besides yellow offspring *either* black or agouti young but not both. This is, of course, evidence that yellow, agouti and black[4] are all allelomorphic to one another.

But the evidence proves nothing of the sort, unless Cuénot had shown that his albinos should have been expected from their history to contain all three factors in question. However likely it may seem, to one so inclined, that such triple forms must sooner or later have been met with by chance, the fact remains that Cuénot had, as the offspring showed, used only double types, and such a fact in the absence of explicit evidence as to the history of the forms used can not be said to *demonstrate* anything in particular.

2. Little continues:

> At the same time he gives the ratios produced by crossing an albino

[3] The "examples" given on page vii of the second memoir are also instructive in the present case.

[4] Probably Little means here by "black" what he later calls the non-agouti factor. If so he refers to a different factor from that which he called black, when, in 1913, he wrote:—"Black . . . is, moreover, clearly a positive character which is dominant over its absence."

potentially a *heterozygous gray* (agouti) with a yellow carrying black but no agouti and albinism. . . . Cuénot recognized that the ratio expected from the cross was 2 yellow, 1 black and 1 agouti (gray).

If one turns to Cuénot's experiment[5] he finds that Cuénot crossed an albino carrying black (AN) to an albino carrying gray (AG) in order to obtain a "dozen" white mice with the formula AGAN, and similarly he crossed a black mouse (CN) to a white mouse carrying yellow (AJ) in order to obtain another "dozen" mice with the formula CNAJ. We are not told whether each dozen came from the same parents, or from several similar combinations. It will be observed that the yellow (J) and black (N) were brought together to make one F_1, and that gray and black were brought together to make the other F_1, hence since gray was in one F_1 and yellow in the other F_1 it is not possible to tell whether they behave as allelomorphs to each other. There is no reason, then, for making gray and yellow both allelomorphic to the same factor (N), black; for, in the first cross the gametes (omitting A and C) might have been Gj (gray) and gj (black) and in the other cross gJ (yellow) and gj (black). The numerical results would then be those obtained by Cuénot, which would prove nothing in regard to the allelomorphism of gray, yellow and black. In other words, the letter N stands in this cross simply as a symbol for anything in the black mouse that could be treated as allelomorphic to G in the one case, and to J in the other; just as at first when rose comb in fowls was found to give a 3 to 1 ratio with single comb it was treated as an allelomorph to single; and likewise when pea was found to give 3 to 1 with single it too was regarded as allelomorphic to single. Later it was found that S (single) stood for two factors ("absences"), small r and small p.

3. Little thinks that both Cuénot and I have fallen into the same error in regard to black; but he fails to see that from our points of view in regard to the other colors it was inevitable that we should come independently to the same conclusion. Little says that the "true" series of allelomorphs is yellow, white-bellied gray, gray-bellied gray, and non-agouti (not "black"). The factor that Little prefers to call non-agouti, I call the black factor. He regards a non-factor as a member of an allelomorphic series, while I regard the black mouse as the result of the action of a factor for black. By the same criterion as that by which Little speaks of a non-agouti factor, he might equally well claim

[5] Third note, p. xlix.

that the "true" series is black, gray gray belly, gray white belly and non-agouti (instead of yellow).

The race of white-bellied mice that I have kept for several years does not correspond in all respects to Cuénot's description of those that he has studied. His account of them in 1908[6] is as follows:

La Souris reste grise sur le dos, mais le ventre prend une teinte blanc roussatre, avec un bouquet de poils plus roux entre les deux pattes de devant, et une bordure un peu plus rousse sur les flancs; elle resemble d'une façon frappante à Mus sylvaticus, L.

Again in 1911[7] Cuénot says:

La première diffère de la Souris grise sauvage par la teinte du ventre, qui, au lieu d'être gris-clair, est blanc roussâtre, avec un bouquet de poils roux entre les deux pattes de devant et une bordure un peu plus rousse sur les flancs; cette Souris a souvent de gros yeux saillants, de sorte qu'elle ressemble d'une façon frappante au Mulot (Mus sylvaticus, L.).

In my race of white-bellied mice there is not a bouquet of russet hairs between the front legs, and I have not observed that the eyes are large and protruding more than occurs at times in other mice. At present, however, I have two old mice that were recently found that have a tuft of faint russet hairs between the forelegs. Whether we have here still another allelomorph, or whether a particular genetic constitution makes apparent the bouquet in conjunction with the white-bellied factor, remains to be worked out. While it seems probable that Cuénot's type of white-bellied mouse and that which I have studied are the same, it is not *certain* that such is the case until further work has been done.

Cuénot has not published as yet any conclusive evidence to show that the gray mice with white belly belong to the series of allelomorphs, although it is true he states that this type is allelomorphic to the three other types. Finally, I should like to add that I am far from wishing to appear to minimize the importance of Cuénot's work, and it is now evident that he should have received full credit for his recognition of the allelomorphic nature of the four factors in question. I still think, nevertheless,

6 Sixth note, II, p. xv.

7 Seventh note, p. xlvii.

that there was room, as matters stood, for the analysis that Sturtevant published and for the work that I carried out.[8]

T. H. Morgan

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A METHOD OF CALCULATING THE PERCENTAGE OF RECESSIVES FROM INCOMPLETE DATA

In the very interesting article on "The Inheritance of Left-handedness" by Professor Ramaley in the December number of the Naturalist, a table is given on page 736 showing the proportion of right- and left-handed children in families where both parents are presumably heterozygous for right- and left-handedness, including only families with left-handed children. In 93 such families there are 282 right-handed and 116 left-handed children.[1] This gives 29.13 per cent. of left-handedness in these families. It is clear, however, that this does not represent the

Total Number of Children from Parents Heterozygous for Right- and Left-handedness, based on the Number of Families Omitted because of Absence of Left-handed Children in Prof. Ramaley's Table 4.

Children per Family	Actual Number of Families	Actual Number of Children	Corrected Number of Families	Corrected Number of Children
1	4	4	16	16
2	14	28	32	64
3	17	51	29 4	88.2
4	23	92	33.63	134.5
5	18	90	23.6	118.0
6	5	30	6.08	36.45
7	1	7	1.154	8.08
8	6	48	6.666	53.35
9	4	36	4.325	38.90
12	1	12	1.032	12.39
		Total		569.87

true Mendelian proportions if right-handedness is a simple Mendelian dominant over left-handedness. For instance, in families where both parents are heterozygous and in which there is only one offspring, the probabilities are that only one family in four will show the recessive character. From the total population re-

[8] *Archiv. de Zool. Exp. et Gen.* (4), IX; (5), VIII.

[1] By oversight in Professor Ramaley's additions one column of right-handed children was omitted, so that the numbers given in the table are incorrect.

sulting from such matings, we therefore leave out three fourths of the families when we include only those showing the recessive character in the offspring. In families of two children nine sixteenths of the families are omitted. In general. the number of families omitted in such a study is $3^n/4^n$, where n is the number of children per family. In order to get the true Mendelian proportions we must take account of these omitted families. The accompanying table shows the most probable results in Professor Ramaley's study had he been able to include the proper proportions of families in which left-handed children might have occurred.

Thus if Professor Ramaley had had at his disposal the full number of families of this character there should have been about 570 children in them, 116 of which were left-handed, or 20.37 per cent.

This is somewhat lower than the theoretical 25 per cent., and I would suggest as a possible cause of this the fact that so many children who are naturally left-handed are from early infancy trained to be right-handed. Hence the number of left-handed children reported is probably less than the true number of recessives in these families.

W. J. SPILLMAN

U. S. DEPARTMENT OF AGRICULTURE

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Devoted to the Advancement of the Biological Sciences with Special Reference to the Factors of Evolution

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THE RÔLE OF THE ENVIRONMENT IN THE REALIZATION OF A SEX-LINKED MENDELIAN CHARACTER IN DROSOPHILA

PROFESSOR T. H. MORGAN

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INTRODUCTION

THE mutant, from which the stock with "abnormal abdomen" was derived, appeared in 1910. It is characterized by a peculiar condition of the pigment bands and segments of the abdomen as shown in Fig. 1. The range of variation of the character is very great; in its most

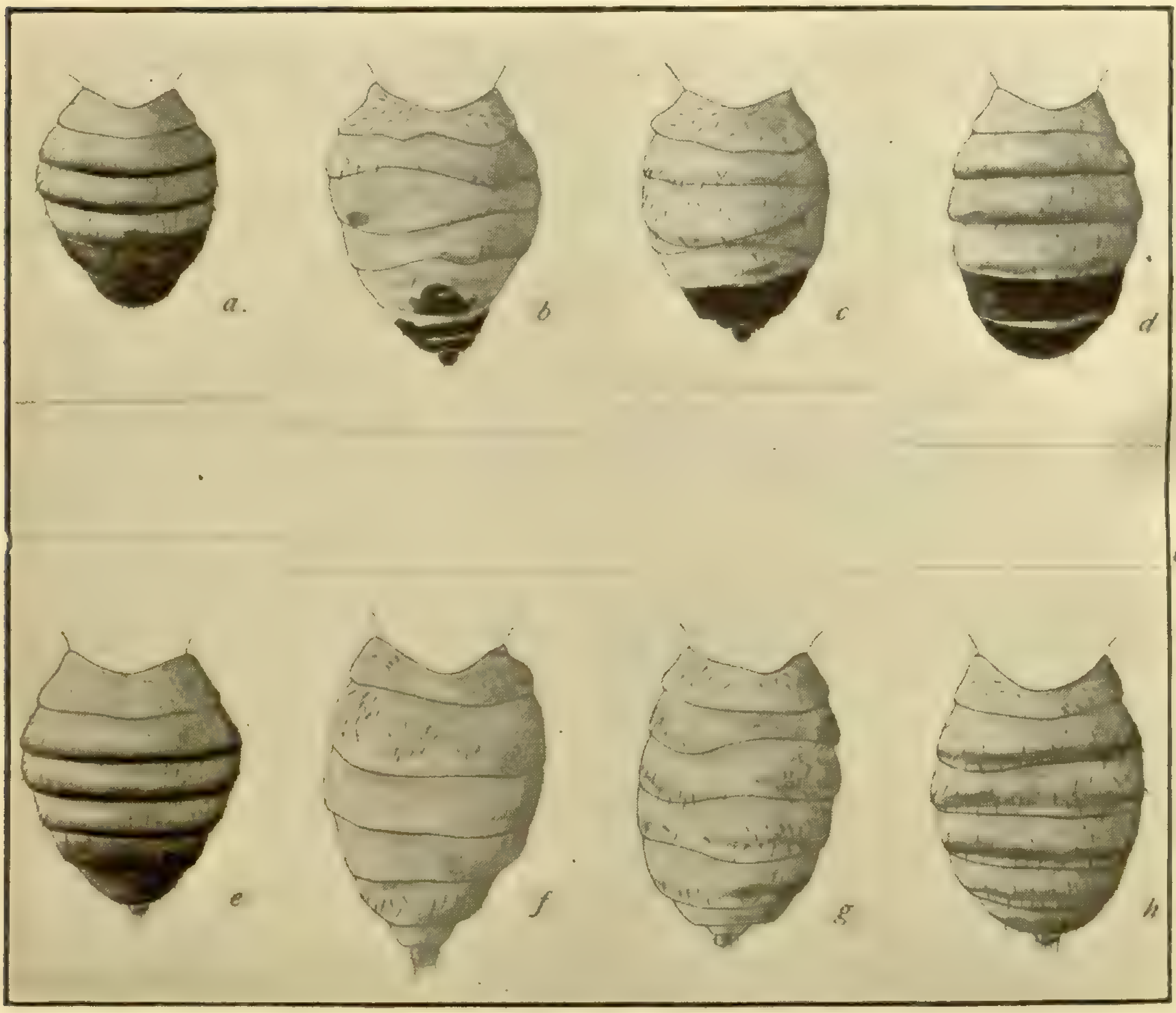

FIG. 1.

extreme condition not only do the pigment bands totally disappear, but even the lines between the metameres are broken up, and the location of the external genitalia may be shifted to a more terminal position. All stages exist between this extreme modification and a condition that can not be distinguished from the normal. Owing to this wide range of variability the study of the inheritance was very difficult until it was found that the realization of the type is a function of the environment.

In the more extreme types the abdomen is deformed to such an extent that copulation is difficult or impossible. The sterility caused in this way helped also to make the work burdensome, especially when breeding was made with pairs. Instead of pairs, cultures of ten to twenty individuals of the more extreme type were resorted to, as a rule insuring the successful mating of some individuals. Aside from this mechanical difficulty in mating, the mutant race is quite vigorous and of good size.

Two principal obstacles delayed the formation of a pure strain. The new character is a sex-linked dominant,[1] but both the heterozygous and the homozygous condition overlap the normal type which makes the selection of pure females difficult. Any male, however, that shows abnormal abdomen at all is pure, for the character is borne by the X chromosomes of which he has but one.

The other obstacle was what at first appeared to be a perpetual reversion of stock, seemingly pure, to the normal. So constantly did this occur, that, for some time, I thought that I had an "ever-sporting" variety—one that reverted to the normal without apparent provocation. I found, however, that the first flies that hatched in the best-fed cultures were entirely abnormal, while those that emerged later were less abnormal, until finally those that emerged when the cultures were nearly at an end were invariably normal flies. It seemed at first possible that such stock might be impure, and that the abnormal flies hatched sooner than the normal, but this view was negatived by the fact that normals hatch as soon as do the abnormal flies.

The one remaining possibility seemed to be that development of the abnormal abdomen depended on some definite condition of the culture—one that was present when the food was fresh and the bottle wet, but which disappeared as the food was used up and the bottle got dry. I tested this hypothesis in many ways. Stock was used that had been pure for nine generations. As a bottle dried up an ever increasing proportion of normal flies appeared. At intervals lots of flies were taken out and put into new bottles where they were abundantly fed. Their first progeny, as recorded below, shows that under the new conditions the offspring were sometimes extremely abnormal irrespective of the general condition of the original stock when used.

[1] Morgan, T. H., "A Dominant Sex-Linked Character," *Proceed. Soc. Exp. Biol. and Medicine*, IX, October 18, 1911.

	Condition of Parents	Next Generation
Feb. 26.	Most flies abnormal—a few were normal	Very abnormal.
Feb. 27.	More than half were normal	Flies fairly normal.
Feb. 28.	About half were normal	Nearly all abnormal.
Feb. 29.	Practically normal	Very abnormal.
Mch. 1.	Nearly all normal	Abnormal, a few normal.
Mch. 4.	Normal	Very abnormal.
Mch. 5.	Normal.	Very abnormal.

The preceding case shows that there is no necessary relation between the development of the abnormality in the parent and that in the offspring. This is only a sample of a large amount of similar data. But this evidence does not show what special conditions make for abnormality. In order to study this problem I generally used heterozygous females which were obtained either by mating an abnormal male to a wild (virgin) female (in which case the daughters will be abnormal under suitable conditions and the sons normal), or reciprocally by mating a normal male to an abnormal female (when all the daughters will be abnormal (heterozygous) and all the sons pure abnormal). Many experiments had shown that the heterozygous female changes over more promptly to the normal character than does the homozygous male and the latter sooner than the homozygous female.

The one outstanding fact for some time was that as a bottle crowded with flies gets old there is always a change from day to day from abnormal towards normal, but it remained to be shown whether the change was due to the drying out of the culture, or to any one of a dozen other parallel changes that obviously are going on at the same time. The more significant results of a prolonged set of experiments may be summed up as follows:

1. *Starvation.*—Lack of food does not bring about the change from abnormal to normal. Flies that are so starved as to be extremely small may be very abnormal.

2. *Acid, Alkali or Neutral Condition of Food Stuff.*—Most cultures change in the course of the ten to twelve days from an acid through a neutral to an alkaline condition. Fresh fermenting banana (in the old and acid

medium) was made more acid (and liquid) by adding an equal amount of a 5 per cent. solution of acetic acid. Other food was made alkaline by adding dry sodium bicarbonate, or a 1 per cent. solution of sodium hydroxide. The acid food gave very abnormal flies; the alkaline food was difficult to control as the flies refused in most cases to lay eggs on it, if it remained alkaline, and the food often dried up, or putrified, or grew mouldy. Moreover, the highly alkaline food often became acid over night owing to fermentation changes taking place within the pieces of fruit used for food. But several times good results were obtained with cultures that had been strictly neutral and often alkaline throughout the time of the experiment and from these the flies were abnormal. Omitting all details it may be stated that an acid or alkaline (neutral) condition as such is not the cause that conditions the character.

3. *Food of Parents.*—At one time it seemed possible that the kind of food that the female was supplied with might for a time continue to affect her eggs, even although the parent was transferred to a medium that acted in the opposite direction. Careful tests showed conclusively that such was not the case. Some of the evidence for this statement will be given later.

4. *Egg versus Sperm.*—Heterozygous females may be produced either by using a normal female and abnormal male, or conversely an abnormal female and a normal male. Certain cultures seemed, at one time, to show that when the egg parent was abnormal the offspring were more abnormal than when the egg parent was normal, but careful tests disproved this view. The difference in the cultures, that led to the suspicion mentioned, was due to the large number of eggs laid by the normal females, hence greater crowding and more rapid disappearance of the moist food.

5. *Influence of Genetic Factors.*—Certain mutant stocks, notably black, seemed at times to show the abnormality less strongly than other stocks, but here, as in the last

case, the results were found to be due, when carefully tested, to the number of eggs laid and the promptitude with which they are laid when the food is fresh. The question will again come up in certain of the crosses.

6. *Amount of Water in Food.*—Normal cultures lose much of their water as the brood of flies develops. It was a fact noticed at the start that in "wet" bottles the abnormal characters appeared to best advantage, and in most of the work on linkage that knowledge was utilized. But whether the wetness was only incidental to other changes, or in itself the normal condition was not precisely determined. Under all conditions the air in the bottles must be completely saturated with moisture so that we must be dealing with the water taken in with the food and not with the amount of water in the inspired air. In three ways the effect of water was studied. (1) Food that had been fermenting for two or three days in the old acid medium was squeezed until freed of much of its water. The solid part was then further dried superficially by pressing between pieces of filter paper, and finally put into a bottle with more dry filter paper. The fluid squeezed out was diluted with an equal amount of water, and put into another bottle. Virgin normal flies and abnormal males of pure stock were set free in these two bottles. The results at the end of nine days were most striking. In the dry bottles the F_1 females were all normal; in the wet bottles the F_1 females were extremely abnormal.

7. *Changing the Adult from Wet to Dry and Vice Versa.*—In this same series the old (P_1) flies that had been in the wet bottle were transferred to dry food, and conversely the "dry" flies to wet food. Their progeny showed the influence of the food that they were reared upon, and no effect of the feeding in the previous bottle. Once more they were changed, the wet to dry, the dry to wet, and the results were the same as before, *i. e.,* the actual conditions, not the preceding ones, fully accounted for the results that were obtained.

8. A culture that was giving F_1 normal females (that were heterozygous for abnormal) was made extremely wet; into a sort of swamp. The flies that emerged during the next six days were normal, on the seventh day the flies were slightly to fairly abnormal, on the eighth and ninth days the flies that emerged were slightly to quite abnormal. It is evident that the influence of the wet conditions does not appear unless the flies are subjected to it throughout most of the larval life, or else that the first few days of larval life is the critical period.

9. Larvæ that were about ready to pupate were transferred to very wet new food, where they pupated, in from 12 to 24 hours. The pupæ remained in the same bottles until the flies emerged. These flies were entirely normal in appearance; the stock from which the larvæ came were also giving rise to normal flies. The sojourn of one or two or even three days in a wet environment at the end of the larval life does not suffice to alter the effects that have already been induced in an earlier stage.

Conclusions.—The preceding evidence makes clear that the amount of water in the food, determines the realization of the "abnormal" type. The water may produce its effect either by being taken in with the food, or by being directly absorbed; or it may determine the nature of the bacterial or yeast flora that in turn determines the nature of the fermentative changes that take place within or without the larvæ. It would be a very difficult matter to find out in which one of these ways the effects are brought about. However this may be, it is possible for the experimenter to determine at will the nature of the flies that will be produced in his cultures by controlling the food supply.

THE LINKAGE OF THE FACTOR FOR ABNORMAL ABDOMEN WITH OTHER SEX-LINKED FACTORS

Owing to the overlapping of the abnormal and normal types, the study of the linkage has presented unusual difficulties. The following experiments were made for the most part during the second year when the influence

of the environment was not fully under control. The conditions under which the experiments were made were, however, favorable for the appearance of the abnormal condition, at least in the first counts of each brood, for the bottles were supplied with an abundance of wet fermented food.

The linkage of abnormal abdomen with white eyes and yellow body color was studied in different combinations; and since the factor for abnormal abdomen proved to be quite near the other two factors the choice was a favorable one in certain respects. A special method by means of which the error, due to the variability of the character, can be largely eliminated will be given after the evidence has been presented.

The Linkage of Abnormal and White

When red-eyed (R) abnormal (Ab) females were mated to white-eyed (W) normal (N) males, red abnormal males and females were produced.[2] When these were mated the results recorded in the next table were obtained.

By means of the following diagram, I have tried to

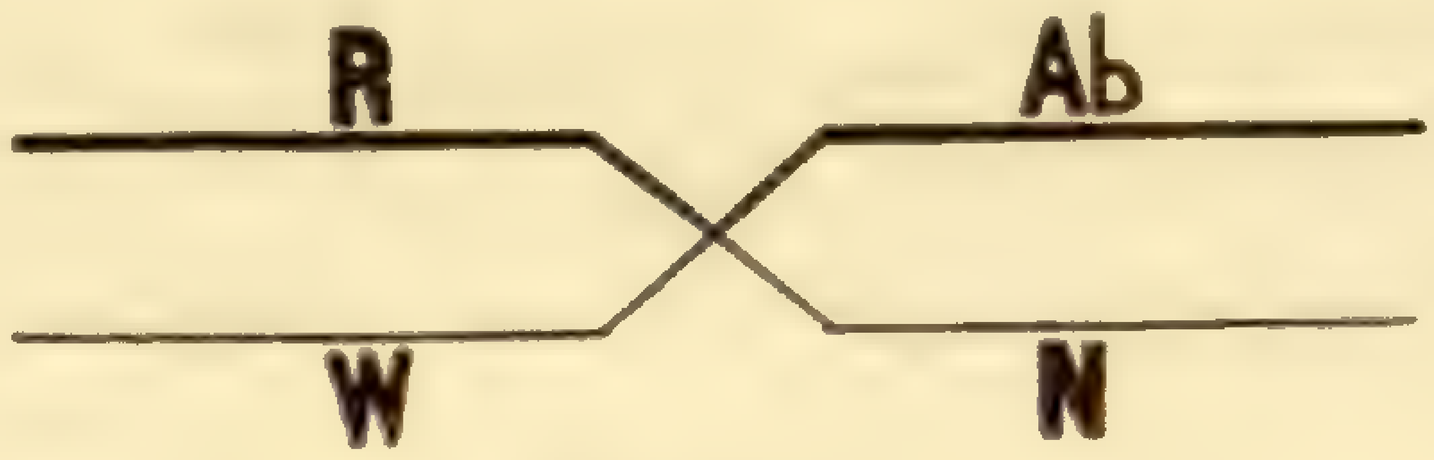

DIAGRAM I.

show what the expectation is for this combination. The two parallel lines are intended to represent the two sex chromosomes of the F_1 female. From her mother she

[2] Throughout this paper I have used the letters R for red eyes, W for white-eyes, N for normal abdomen, Ab for abnormal abdomen, Y for yellow, B for black instead of using the allelomorphic system; because for present purposes, where analyses are unnecessary, these letters suffice most simply to indicate the operations that are involved. For comparison with other papers the allelomorphic symbols for the same characters would be:

w = the factor for white. W = its normal allelomorph = red.
A'b = the factor for abn. abd. a'b = its normal allelomorph = normal abd.
y = the factor for yellow. Y = its normal allelomorph = gray.
b = the factor for black. B = its normal allelomorph = gray.

got the sex chromosome bearing the factors for red and abnormal (RAb), from her father the homologous sex chromosome that carries the factors for white and normal (WN).

TABLE I

PARENTS: RAb ♀ BY WN ♂

F_1: RAb ♀—RAb ♂

RAb		WN		RN		WAb		No. of Culture
♂	♀	♂	♀	♂	♀	♂	♀	
157	336	157	......	9	2	5	......	II_1
143	266	154	......	6	1	3	......	II_4
106	362	130	......	2	3	1	......	II_5
88	151	62	......	4	......	2	1	II_{16}
95	200	100	......	2	......	1	......	II_{20a}
24	68	41	......	2	......	......	......	II_{28}
34	64	20	......	......	1	1	......	II_{29}
647	1,447	664	......	25	7	13	1[3]	

If these chromosomes unite at synapsis without exchange of materials, half of the eggs that result (one chromosome being eliminated in the polar bodies) will contain the red normal combination, the other half the white normal. These represent the "non-cross-over" gametes. If, however, these chromosomes should cross and reunite, as in the diagram (the crossed lines indicate where the crossing over may occur, not how it occurs), the two resulting chromosomes will be red-normal RN, and white abnormal, WAb, which represent the other (the cross-over) kinds of gametes of the F_1 female. The ratio in which they are produced is the gametic ratio and is a measure of the linkage.

In the F_1 males there is but one X chromosome, hence there is no opportunity for interchange here between the X chromosomes. The mate of the X chromosome is, in the male, the Y chromosome. Other experiments have shown that the Y chromosome carries no factors; hence interchange seems precluded; and, so far, no loss of X chromosome factors to the Y chromosome has ever been observed. The X chromosome passes into the female-

[3] An unexpected individual that can be accounted for by equational non-disjunction.

producing spermatozoon, which carries therefore an X chromosome received from the mother of the F_1 males and bears her character. In the present case the male carries the chromosome bearing red abnormal.

Since red and abnormal dominate, all the F_2 females should be red abnormal, except in so far as the conditions suppress the abnormal and induce the normal type. The experiment, Table I, shows that very few normal females were present.

Four classes of males are expected—the large class of non-cross-overs RAb and WN, and two small classes of cross-overs RN and WAb. It will be observed (Table I) that the linkage between R and Ab is very strong, since nearly all of the males are either RAb (647) or NW (664). Only a few crossovers RN (25) and WAb (13) males were present. The percentage of crossing over is 1.97 per cent. when the abnormal males alone are used for calculation.

In the reciprocal cross the RAb male was mated to WN female, and gave in F_1 RAb females and WN males. The F_2 record is given in Table II.

TABLE II

PARENTS: RAb ♂ BY WN ♀

F_1: RAb ♀—WN ♂

RAb		WN		RN		WAb		
♂	♀	♂	♀	♂	♀	♂	♀	
68	62	59	47	1	0	2	1	II_3
38	55	69	68	0	3	0	1	II_{20}
115	170	130	147	2	2	2	2	II_{21}
103	103	97	103	3	7	4	2	II_{22}
75	96	94	49	2	1	5	0	I_{13}
399	486	449	194	8	13	13	6	

Since the same two pairs of factors enter as before, the same chromosome diagram will suffice for the gametes of the F_1 female. The F_1 male is, however, a double recessive (WN); in consequence four classes of females are expected as well as of males. The gametes of the F_1 female are as before the following:

Non-cross-over gametes	RAb WN	Crossover gametes	RN WAb

The percentage of crossing over as calculated from the abnormal classes (males and females) is 2.1.

In order to obtain further data for linkage the preceding experiment was repeated in the winter of 1914, but the linked factors entered differently combined. The experiment was begun by crossing white abnormal females to wild males which gave red abnormal females and white abnormal males. These were inbred and gave the following results in five different cultures (kept with abundance of moist food).

TABLE III

PARENTS: WAb ♀ BY RN ♂

F_1: RAb ♀—WAb ♂

No.	RAb		WN		RN		WAb	
	♂	♀	♂	♀	♂	♀	♂	♀
1	0	44	0	2	33	0	52	55
2	4	66	0	0	57	4	57	50
3	1	38	0	0	62	0	37	48
4	1	111	7	1	95	0	77	100
5	1	65	2	0	65	0	28	34
Total	7	324	9	3	312	4	251	287

The sum of the two non-cross-over males ($251 + 312 = 563$) plus the cross-overs (16) divided into the sum of the cross-over males ($7 + 9 = 16$) gives 2.7 as the percentage of crossing over. Since the white normal males may receive contributions from the changed white abnormal, the result may be freer from error if the two correlative abnormal male classes, viz., red abnormal (7) and white abnormal (251), be utilized to calculate crossing over. Dividing the former by the total ($251 + 7$) gives 2.7 per cent. of crossing over which is the same as the preceding estimate.

The reciprocal cross, RN ♀ by WAb ♂, was also made once and the results in F_2 combined with other similar results are as follows:

RN ♂	♀	WAb ♂	♂	RAb ♀	WN ♂
1,220	(with RAb)	854	20	1,323	89

The other results were obtained in the following way: The abnormal red eyed F_2 females obtained from the first experiment are heterozygous for abnormal (AbN) and white (RW), except in so far as this class may contain cross-over flies that are heterozygous in white but homozygous in abnormal AbWAbR. Except for these flies, these F_2 females are like the F_1 females, and if mated to abnormal white males will continue in each successive generation to give the same linkage data as do the F_2 classes above. If bred in pairs exceptional females homozygous for abnormal will be at once detected, and can be thrown out; but even if bred in small batches of four or five females the chance is small of including homozygous abnormal females.

In these counts no separation of the normal red females (when they occurred) from the abnormal red females was made but the red females were put into the latter class. Since the females were not intended to be used for comparison this grouping does not affect the problem involved. If we divide the cross-over red abnormal males (20) by the abnormal white males (854) plus 20 abnormal red males, we get the per cent. of cross-overs which is here 2.3. This is slightly lower than that obtained for the preceding data.

Black, Red, Abnormal by Gray, White, Normal

Another series of experiments, carried on for a somewhat different purpose, may be utilized here for further data. Gray, white, normal females were mated to black, red, abnormal males. The daughters were gray, red, normal (or slightly abnormal), and the sons gray, white, normal. Inbred they give the results shown in Table IV. Since the factor for black is not sex-linked, the gray and the black classes may be added together as shown in Table V.

The results differ from those of Table II in the following points: There are relatively more red normals which may be assumed to be due to the external condition pre-

TABLE IV

PARENTS: BRAb ♂ BY GWN ♀

F_1: GRN OR SLIGHTLY Ab ♀—GWN ♂

GRAb		BRAb		GWN		BWN		GRN		BRN		GWAb		
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
35	52	7	8	160	179	48	58	126	146	54	43	...	...	III_5
50	78	6	16	111	104	34	18	48	30	34	28	...	...	III_{29}
65	52	0	1	221	187	13	7	131	159	11	16	1	...	III_{35}
187	152	27	36	254	268	58	72	102	99	45	65	4	1	III_{48}
33	56	12	12	37	63	15	10	0	0	0	0	...	...	III_{49}
103	140	34	34	146	191	52	42	54	52	21	14	6	1	III_{50}
50	33	14	10	93	69	22	24	30	24	7	6	1	...	III_{52}
8	71	0	2	76	123	20	40	80	66	27	61	...	...	III_{59}
91	74	17	16	89	89	30	45	75	66	79	59	1	...	III_{69}
92	88	27	24	135	153	33	59	62	76	19	20	...	1	III_{89}
2	8	6	3	7	8	3	3	0	0	0	0	...	...	Ex.
82	95	32	23	195	191	47	65	66	94	34	29	...	1	III_{19}
798	899	182	185	1,524	1,625	375	443	774	812	331	341	13	4	

vailing at the time, or else the black factor may have had some influence that is favorable to the normal condition in the heterozygous abnormal flies. If the latter were the true explanation we can understand the large number (here) of the GRN class (for two thirds are heterozygous in black and intermediate in color) and the complete absence of the BWAb class which should be one third as frequent as the GWAb class. A special examination was made to test this possibility and will be described

TABLE V

PARENTS: BRAb ♂ BY GWN ♀

F_1: GRN OR SLIGHTLY Ab ♀—GWN ♂

RAb		WN		RN		WAb		
♂	♀	♂	♀	♂	♀	♂	♀	
42	60	208	237	179	189	......	......	III_5
56	94	145	122	82	58	......	......	III_{29}
65	53	234	194	142	175	1	......	III_{35}
214	188	312	340	148	164	4	1	III_{43}
45	68	52	73	....	....	......	......	III_{49}
137	192	198	233	75	66	6	1	III_{50}
64	43	115	93	37	30	1	......	III_{52}
8	73	96	163	107	127	......	......	III_{59}
108	90	119	134	154	125	1	......	III_{69}
119	112	168	212	81	96	......	1	III_{89}
8	11	10	11	....	....	......	......	Extra
105	118	242	256	100	123	......	1	III_{15}
841	1,102	1,899	2,068	1,105	1,153	13	4	

later, but it may be stated beforehand that no certain evidence could be found in favor of this view. The number of larvæ in a culture brings about a rapid alteration in the condition of the food, so that it changes more quicky from an acid to a neutral or alkaline condition. If the black flies used gave vigorous F_1 offspring the effect in question could be explained as due to numbers, and not as connected with the black factor.

Gray, Red, Abnormal by Black, White, Normal

The results of this cross and of its reciprocal are given in Tables VI and VII. The RN class (cross-over) is relatively too large, but the increase is due to the transition from abnormal to normal.

TABLE VI

Parents: GRAb ♀ by BWN ♂

F_1: GRAb ♀—GRAb ♂

GRAb		BWN		GWN		BRAb		GRN		BWAb		GWAb		BRN	
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
95	194	49	2	163	1	20	32	56	71	...	...	4	.....	24	45
Parents: BWN ♀ by GRAb ♂ — F_1: GRAb ♂—GWN ♀															
215	143	91	91	314	276	56	11	28	170	...	...	9	2	41	59

THE LINKAGE OF ABNORMAL, YELLOW, AND WHITE

In the following crosses three pairs of sex-linked factors characters are involved, viz., yellow, white, abnormal and their normal allelomorphs whose location at one end of the X chromosome is shown in Diagram II.

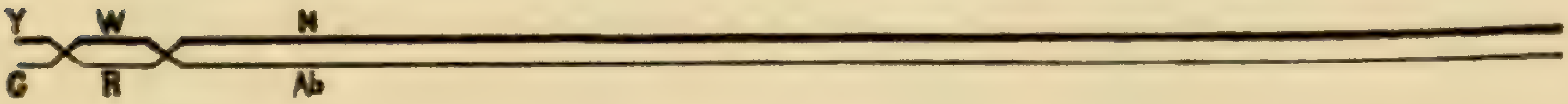

Diagram II.

Gray, Red, Abnormal by Yellow, White, Normal

When a YWN ♀ is crossed to a GRAb ♂ the daughters are GRAb and the sons YWN. The F_1 male is a triple re-

cessive, hence, neither his female-producing nor his male-producing sperm affect the dominant characters that the eggs carry, and in consequence the entire F_2 count, females as well as males, are indicators of the gametic composition of the eggs of the F_1 female. The F_2 results are given in Table VII.

TABLE VII

PARENTS: GRAb ♂ BY YWN ♀

F_1: GRAb ♀—YWN ♂

	YWN		GRAb		YRAb		GWN		YWAb		GRN		YRN		GWAb		
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
1	45	59	44	51	...	...	...	...	...	...	6	3	...	...	...	...	I_1
2	83	69	59	53	...	...	...	...	...	...	19	31	...	...	...	...	I_5
3	115	107	132	150	1	2	...	...	6	5	4	7	...	...	...	...	I_7
4	174	177	152	205	...	2	5	2	4	...	6	9	...	1	...	...	I_{10}
5	100	128	108	150	...	7	1	2	5	3	2	9	...	...	...	...	I_{11}
6	42	52	58	40	...	2	1	2	3	...	1	1	...	...	...	...	II_3
7	92	94	105	114	...	17	1	1	1	1	12	...	...	1	...	...	II_6
8	105	130	123	97	...	...	2	4	1	...	3	4	...	3	...	...	II_8
9	83	78	92	99	...	...	1	1	...	...	10	5	...	...	...	...	II_9
10	83	81	90	107	...	1	...	...	2	...	6	15	...	...	...	...	II_{10}
11	375	374	441	443	1	6	4	3	5	7	22	14	...	...	...	...	II_{11}
12	103	127	125	136	1	...	...	...	2	...	7	8	1	...	...	...	II_{12}
13	135	116	119	169	1	1	...	1	...	...	9	8	...	...	...	...	II_{13}
14	101	92	116	105	1	1	...	...	...	...	4	20	...	...	...	...	II_{15}
15	29	56	45	63	1	...	1	...	1	...	1	3	...	...	...	...	II_{18}
16	45	58	50	77	...	...	...	4	...	1	2	...	...	...	...	...	II_{19}
17	33	37	27	40	1	1	...	...	1	...	2	1	...	...	...	...	II_{24}
18	31	45	30	36	1	...	...	1	...	1	1	2	...	...	...	1	II_{25}
19	236	231	283	276	6	2	4	3	9	1	6	6	...	1	...	...	II_{27}
20	47	63	31	62	1	...	...	...	...	...	2	4	...	...	...	...	II_{30}
21	66	79	101	64	...	...	2	4	...	...	17	52	...	...	...	...	II_{31}
22	325	307	209	250	1	1	3	2	1	...	139	251	2	1	...	...	IV_4
23	286	184	233	321	6	2	5	3	2	2	197	191	1	1	...	1	IV_5
	2,734	2,744	2,773	3,008	22	45	30	33	43	21	478	644	4	8	0	2	

The relation of the classes to each other is evident from the following diagram (III) which represents (as before)

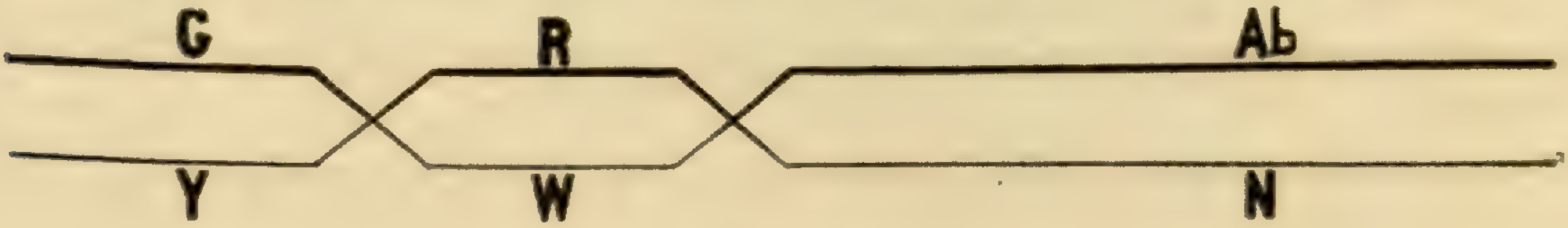

DIAGRAM III.

the sex chromosomes of the F_1 female. The classes of gametes of the F_1 females are the following:

Non-Crossovers	Single Crossovers	Double Crossovers
YWN	YRAb	YRN
RGAb	GWN	GWAb
	YWN	
	GRN	

In this and in the following tables the order of the cross-over gametes is always given the same, viz.: the first factor to the left above (Y) joins the two following below, R and Ab, (taking the switch as it were at the first cross-over). Then follows the cross-over that is the converse of the preceding (the first factor to the left below switching over to join W and N). The second crossing is taken in the same way, thus Y and W switch over to Ab, and conversely G and R switch over to N. The double cross-over takes the switch twice; thus Y to R and then to N; and conversely G to W and then to Ab. The F_2 flies should correspond to these gametic classes (since the F_1 male was a triple recessive) except in so far as the abnormal classes change to phenotypic normal types. Thus the non-cross-over class GRAb will, in this sense, contribute to the single cross-over class GRN; and the single cross-over class YRAb to the double cross-over class YRN. The last-named class can not, therefore, be used as a measure of the double crossing over, since it is more probable that any flies of this kind that appear will be only phenotypic YRN, than that they should belong to the YRN class genetically. Only the GWAb class may be used as a measure of double crossing over, and, as will be shown below, much caution must be used even in this case.

It will be seen in the table that only relatively few of the GRAb type have changed to the normal type, because the conditions were favorable for abnormal although the cultures ran in most cases for ten days, but during this time they still contained plenty of wet food. It will be noticed that the changed class GRN corresponds to one of the single cross-over classes, consequently GRN is a mixed class, and can not be used to base any calculation on. It is true, one may roughly determine how many

cross-overs are expected in this mixed class by comparison with the other single cross-over class (YWAb). If these are subtracted, the remainder shows how many of this GRN class are due to a change from the abnormal to normal. Another point to note is that one of the double cross-over classes, viz., YRN, is likewise subject to addition from the single cross-over class, YRAb, and can not itself be taken as a measure of double crossing over, while, on the contrary, all cases in the other double cross-over class, viz., GWAb, count for their full value. Only two such double cross-overs occurred.

On the basis of the amount of single crossing over it is possible to calculate, as Sturtevant has shown, the expected number of double cross-overs. The number of the double cross-overs (two) in Table VIII is larger than expected. I repeated (December, 1913) the last experiment to test the question because abnormal arrangement of the rings of the abdomen is not a very rare occurrence and may sometimes be the result of injury to the larva or to the pupæ, or in still other cases may be due to other mutations, some of which will be described later. The abnormal mutation itself occurs not infrequently under conditions precluding contamination. In repeating the experiment extreme care was taken not to classify any fly

TABLE VIII

PARENTS: GRAb ♂ BY YWN ♀

F_1: GRAb ♀—YWN ♂

	YWN		GRAb		YRAb		GWN		YWAb		GRN		YRN		GWAb	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
A	58	64	57	56	0	0	0	0	5	0	0	1				
D	59	67	45	63	2	4	1	0	0	3	1	1				
E	72	90	60	76	0	1	0	2	0	1	2	3				
F	97	92	88	71	1	2	1	2	3	2	1	3				
H	31	41	47	58	1	0	0	0	0	0	3	3				
J	37	50	45	55	1	1	1	1	0	1	3	2				
K	64	65	56	52	2	0	1	0	2	0	1	2				
L	83	90	115	120	2	0	1	0	3	1	4	3				
M	64	65	74	75	0	2	0	0	3	3	6	3				
N	40	51	38	57	0	0	0	0	0	1	3	3				
Totals	605	675	625	683	9	10	5	5	16	12	24	24	0	0	0	0

in the double cross-over class as abnormal unless there could be no reasonable doubt as to the nature of the character. In case of doubt the flies were tested by crossing again.

As before, yellow white normal (abdomen) females were crossed to gray red abnormal males. These gave in F_1 YWN ♂ and GRAb ♀ which inbred gave the results shown in Table VIII.

The double cross-over class is GWAb. The combination did not appear once amongst the 2,690 flies that are recorded in F_2. The percentage of crossing over between Y and W is 1.0; that between W and Ab was 2.1. The expectation of double crossing over on this basis (without interference) would be .02 per cent., or about 1 in 5,000. But the expectation would be far smaller than this because of a principle that we call interference. We mean by this term that should a cross-over occur at one point the chance of another occurring near it is greatly diminished, because if crossing over is due to twists of the chromosome the length of a twist would usually preclude the occurrence of two cross-overs near one another. In other words, if the loop that makes the twist is more likely to be of a certain length then the likelihood of the occurrence of a small loop necessary for a double cross-over is very small. In two cases, *B* and *C*, the F_2 counts (from pairs of F_1 flies) gave no YWN males as shown in the next counts.

	YWN		GRAb		YRAb		GWN		YWAb		GRN	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
B	0	55	51	41	1	1	0	0	0	2	1	3
C	0	49	47	47	0	1	0	0	0	2	0	4

The absence of the YWN males, when the other classes showed that no error in the experiment had been made, was not understood until the occurrence of lethal factors was worked out. Here clearly a lethal factor in the YWN grandmother has been carried over into her GRAb daughter. The lethal factor must have been closely

linked with yellow and with white. The F_1 YWN son of the original YWN female must have come from the other sex chromosome of the YWN female—the one that did not carry the lethal. The count of the males in the F_2 gives both in *B* and in *C* a 2:1 ratio which is the characteristic ratio for a sex-linked lethal. The reciprocal cross was also made, but only twice; the F_2 counts are given in Table IX.

TABLE IX

PARENTS: GRAb ♀ BY YWN ♂
F_1: GRN ♀ (OR SLIGHTLY Ab)—GRAb ♂

YWN		GRAb		YRAb		GWN		YWAb		GRN		YRN		GWAb	
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
141	...	165	339	......	...	2	...	1	...	59	110	...	...	...	...
202	...	225	562	3	...	.....	...	7	...	10	11	...	...	1	...

The expected gametes of the F_1 female are the same, of course, as before, but the male contains all three sex-linked dominant factors, GRAb. Consequently in F_2 half of the GRAb female class is pure and half is heterozygous for abnormality. The GRAb F_2 males, on the other hand, are all pure, in the sense that they have only one factor for abnormal and no factor for normal. It is probable that most of them here are phenotypically abnormal.

The relation of the non-cross-over and the cross-over gametes is the same as in the reciprocal cross, since only sex-linked factors are involved, but the cross-over classes given in Table IX are different in the female classes in so far as the female producing sperms, that carry GRAb, contain three dominants. In one of the two counts given in the table the cross-over class that has changed to phenotypic normal is relatively large; in the other count it is small.

Gray, White, Abnormal by Yellow, Red, Normal

The next largest series of experiments involves the same three pairs of characters but combined in a different way. The results are shown in Table X. Diagram IV shows the relative positions of the factors in this combination.

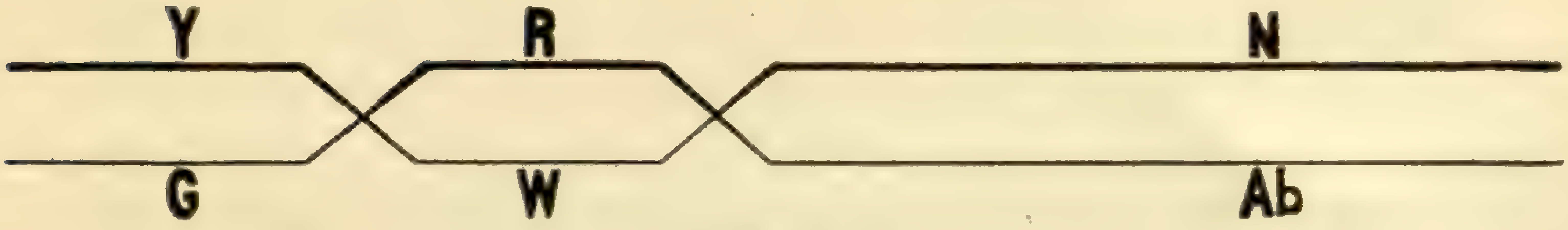

Diagram IV.

The gametes produced by the F_1 female are the following.

Non-Crossover Gametes	Single Crossover Gametes	Double Crossover Gametes
YRN	YWAb	YWN
GWAb	GRN	GRAb
	YRAb	
	GWN	

The classes of special interest are non-cross-over GWAb males which change as the culture gets old into GWN (which is a single cross-over class), and GRN which is the corresponding female class (but heterozygous).

TABLE X

Parents: GWAb ♂ by YRN ♀

F_1: GRAb ♀ (to N)—YRN ♂

YRN		GWAb		YWAb		GRN		YRAb		GWN		YWN		GRAb		
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
3	42	32	...	...	...	...	2	45	1	3	...	...	...	...	67	III$_3$
470	494	329	...	1	...	10	185	1	...	115	...	1	...	...	232	III$_{20}$
92	101	99	...	...	...	...	.....	1	...	17	...	1	...	...	124	III$_{21}$
127	123	50	...	...	...	3	51	...	1	56	...	...	...	...	130	III$_{26}$
184	196	181	...	1	...	7	20	...	...	8	...	...	...	...	239	III$_{26}$
234	286	168	...	...	...	6	155	...	...	69	...	...	...	...	151	III$_{31}$
108	149	80	...	1	...	...	114	...	...	49	...	...	...	...	79	III$_{32}$
321	381	218	...	1	...	2	256	3	1	123	1	1	...	...	259	III$_{37}$
158	167	54	...	1	...	...	105	1	1	104	...	2	...	...	96	III$_{42}$
178	169	60	...	...	...	8	155	2	...	140	...	1	...	...	62	III$_{43}$
185	181	107	...	...	...	1	91	...	...	80	...	1	...	...	119	III$_{54}$
100	109	54	...	...	...	3	20	1	1	28	...	1	...	...	114	III$_{55}$
189	185	141	...	1	...	1	44	...	...	27	...	...	...	...	172	III$_{57}$
332	322	233	...	3	...	10	45	3	1	27	...	4	...	...	309	III$_{64}$
244	279	19	...	...	...	6	324	...	1	123	1	...	...	...	14	III$_{65}$
147	144	98	...	...	...	6	169	...	...	86	...	...	...	...	8	III$_{67}$
236	221	244	...	2	...	1	105	2	...	56	...	1	...	...	241	III$_{68}$
30	25	19	...	...	...	...	31	1	...	3	...	...	...	...	19	III$_{77}$
230	227	97	...	...	...	5	161	...	...	176	...	3	...	...	86	III$_{79}$
106	80	74	...	...	...	1	112	...	1	43	...	3	...	...	30	III$_{90}$
130	115	98	...	...	...	1	81	1	...	54	1	...	...	...	64	III$_{92}$
111	128	82	...	2	...	11	116	...	...	49	2	1	...	2	61	III$_{98}$
65	83	40	...	...	...	1	37	...	...	23	...	...	...	...	47	
225	202	100	...	...	...	2	142	...	...	93	...	...	...	...	94	
4,205	4,409	2,677	...	13	...	85	2,521	61	8	1,552	5	20	...	2	2,817	

An examination of Table VI shows how extensively changing took place in almost every one of the experiments. The contrast with the result of Table V is very striking.

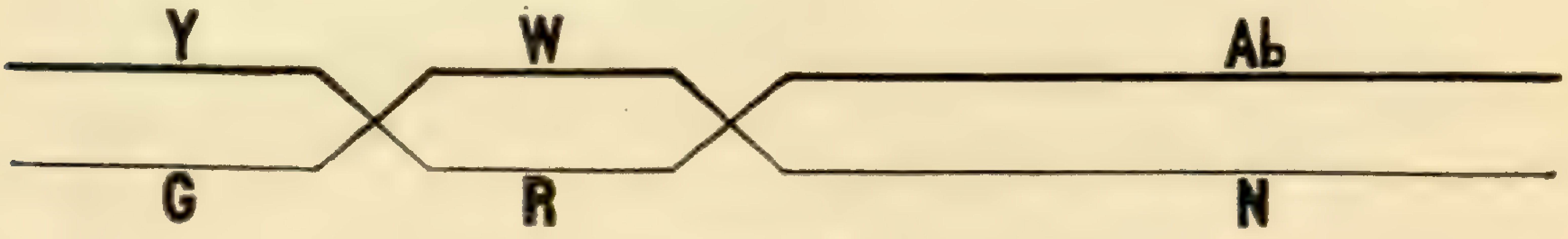

DIAGRAM V.

Yellow, White, Abnormal by Gray, Red, Normal

The experiment was made once one way (Table XI) and seven times reciprocally (Table XII). In the first case

TABLE XI

PARENTS: YWAb ♀ BY GRN ♂
F_1: GRN ♀—YWN ♂

GRAb		YWN		GWN		YRAb		GRN		YWAb		GWAb		YRN		
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
3	105	58	25	1	1	...	2	167	145	74	47	...	3	5	10	III_{23}

nearly all of the GRAb females are of the normal type. The only GR males that are abnormal are single crossovers (Diagram V). This means that the heterozygous females are affected more easily than are the pure males;

TABLE XII

PARENTS: YWAb ♂ BY GRN ♀
F_1: GRN ♀—GRN ♂

GRAb		YWN		GWN		YRAb		GRN		YWAb		GWAb		YRN		
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
		97	...	5	...	...	...	225	536	....	...	...	...	57	...	III_{25}
	25	15	...	...	...	...	...	205	313	28	...	...	...	4	...	III_{29}
1 slight	...	71	1	2	...	...	...	87	202	....	...	...	...	...	...	III_{41}
1	71	33	...	...	...	...	...	198	259	46	...	2	...	...	...	III_{46}
3	69	40	...	5	...	...	...	488	648	59	...	6	...	3	...	III_{58}
		47	...	2	...	...	...	67	113	....	...	...	...	1	...	III_{78}
1	9	5	...	1	...	...	...	219	249	36	...	1	...	1	...	III_{91}
Total 5.	174	308	1	15	...	...	...	1,489	2,320	169	...	9		66	...	......

[4] There is an exceptional case in the table, viz., two GWN ♀.

but even amongst the females a large percentage of gray reds are normal. The yellow white abnormal class is relatively much more abnormal, *i. e.*, relatively fewer have undergone the transition.

The results from the reciprocal cross are given in the next table. Here the F_1 male contains two dominants (GR) and one recessive factor (N). The females GRN YWAb carry only one dose of Ab, yet they are largely abnormal. The GRAb are single cross-overs.

Yellow, Red, Abnormal ♀ by Gray, White, Normal ♂

Only one experiment of this kind was made, but as the number of F_2 flies was rather large the results may be given (Table XIII).

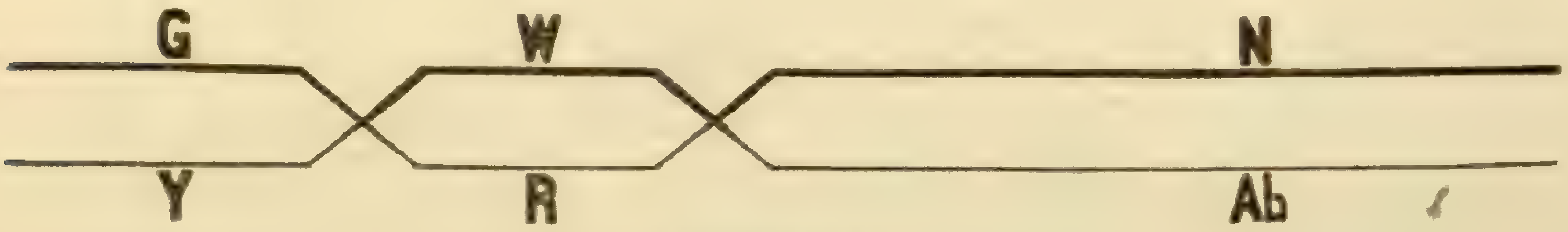

Diagram VI.

TABLE XIII

Parents: YRAb ♂ by GWN ♀

F_1: GRAb ♀—GWN ♂

GWN		YRAb		GRAb		YWN		GWAb		YRN		GRN		YWAb	
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
166	171	135	....	....	154	....	....	2	1	....	....	1	36		
198	215	183	....	....	192	....	....	1	1	....	....	....	31		
210	215	179	....	....	232	2	....	1	1	....	....	....	85		
217	223	165	....	1	140	1	....	3	....	....	....	1	101		
791	824	662	....	....	718	3	....	7	3	....	....	2	253		

In this cross the gametes are as follows:

Non-Cross-over	Single Cross-over	Double Cross-over
GWN	GRAb	GRN
YRAb	YWN	YWAb
	GWAb	
	YRN	

The F_1 male is GWN and contains, therefore, one dominant sex-linked factor, viz., G. Therefore, all of the F_2

females are gray. The F_2 male classes alone can be used for testing the extent of crossing over.

CHANGE OF TYPE AS THE CULTURE GROWS OLDER

The preceding tables do not bring out the change that takes place as the culture gets older—a change by which the abnormal classes become replaced by the normal classes. A few results will therefore be given in detail to illustrate this relation.

In none of the relatively few counts in Tables I, and II, involving two pairs of factors (RN and WAb), was any change in type during the time of the experiment noticed; but in other cases a very marked change was observed as the cultures grew older.

In the two following tables consecutive counts of the F_2 flies (from YRAb ♂ by GWN ♀) emerging from day to day from the same culture are given. The change of the YRAb to the YRN and of GRAb to GRN is very striking.

TABLE XIV

Parents: YRAb ♂ by GWN ♀
F_1: GRAb ♀—GWN ♂

	YRAb		GWN		YWN		GRAb		YRN		GWAb		YWAb		GRN	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
III_2....	81	...	98	88	....	...	...	95	1	...	1	1	...	...	1	....
	47	...	49	49	....	...	...	56	2	...	1	....	...	...	...	4
	7	...	19	34	....	...	...	3	25	...	....	....	...	...	...	32
	135		116	171	....	...	...	154	28	...	2	1	...	...	1	38
III_{74}...	57	...	44	55	1	...	...	83	....	...	....	1	...	...	...	....
	38	...	39	39	1	...	...	43	....	...	....	....	...	...	...	....
	44	...	33	38	....	...	...	64	....	...	1	....	...	...	...	....
	40	...	36	27	....	...	...	36	....	...	....	....	...	...	...	4
	....	...	63	51	....	...	...	6	74	...	....	....	...	...	...	81
	179	...	215	210	2	...	...	232	74	...	1	1	...	...	...	85

In the next table two F_2 counts are given derived from GWN ♀ by BRAb ♂ grandparents. The GRAb changes to GRN and BRAb to BRN.

TABLE XV

PARENTS: BRAb ♂ BY GWN ♀
F_1: GRN ♀ (OR SLIGHTLY Ab)—GWN ♂

Counts	GRAb		GRN		BRAb		BRN		GWAb		GWN		BWAb		BWN		
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
1	23	33	0	13	5	5	0	0	...	...	23	26	...	...	3	6	III₅
2	6	16	0	11	2	3	2	4	...	...	13	9	...	...	2	2	
3	1	0	17	24	0	0	6	7	...	...	15	24	...	...	5	7	
4	0	0	36	40	0	0	15	12	...	...	41	39	...	...	15	22	
5	6	3	76	71	0	0	31	20	...	...	67	81	...	...	23	21	
1	19	24	1	0	11	6	2	0	...	...	23	20	...	...	10	8	III_{10}
2	26	28	0	0	7	10	0	0	...	...	50	29	1	...	9	12	
3	29	30	0	0	8	2	3	0	...	...	28	37	...	...	7	7	
4	8	13	2	0	6	5	0	0	...	...	11	14	...	...	4	4	
5	0	0	27	43	0	0	12	13	...	...	32	42	...	...	7	13	
6	0	0	36	51	0	0	17	16	...	...	51	49	...	...	10	21	

In the next case eight consecutive F_2 counts are given. The GRAb changes to GRN. In the first three counts there are 221 GRAb to 76 GRN, or 3 to 1. In the last three counts there are 148 GRAb to 296 GRN or 1 to 2.

TABLE XVI

PARENTS: GRAb ♂ BY YWN ♀
F_1: GRAb ♀—YWN ♂

YWN		GRAb		YRAb		GWN		YWAb		GRN		YRN		GWAb	
♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
105	90	91	100	0	0	1	1	0	0	15	51	1	1		
20	27	17	19	0	0	0	0	0	0	2	2	0	0		
32	35	33	51	0	0	1	0	0	0	2	4	0	0		
7	5	5	3	0	0	0	0	0	0	0	4	0	0		
5	4	1	1	0	0	0	0	0	0	3	11	0	0		
67	55	14	15	0	0	1	1	1	0	95	127	1	0		
47	28	23	22	0	0	0	0	0	0	18	36	0	0		
42	63	35	39	1	1	0	0	0	0	4	16	0	0		
325	307	219	250	1	1	2	2	1	0	139	251	2	1	0	0

Finally six cultures are given in the following table of F_2 flies from YRN ♀ by GWAb ♂ grandparents. The GWAb males change to GWN males and the GRAb females to GRN females. In both cases the increase in the normal flies in the last two or three counts is marked.

TABLE XVII

PARENTS: GWAb ♂ BY YRN ♀
F_1: GRAb ♀—YRN ♂

	YRN		GWAb		YWAb		GRN		YRAb		GWN		YWN		GRAb	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
III_{20}...	44	43	32					3								46
	36	53	33						1		1					73
	59	68	71					2			4					57
	72	67	56				2				7					35
	84	98	38		1			18			31		1			3
	25	15	20				1	10			9					3
	60	49	49				1	4			6					11
	37	40	28				1	17			17					3
	38	43	2		1						28					
	15	18					1	15			12					
III_{31}...	44	51	35				1	1								80
	36	43	28				1									44
	50	46	46					29			1					24
	29	48	6					42			36					
	75	98	53				4	83			32					3
III_{42}...	28	33	21		1					1						46
	27	24	26						1		8					48
	42	38	2					50			40					2
	42	31	5					29			26					
	19	44						26			30		2			
III_{45}...	26	36	32					6	2		1					39
	25	22	28					47			55					19
	57	41	0					72			76					4
	63	68	0					7			8		1			
	8	2	0				8	23								
III_{64}...	39	21	29				2									
	144	181	126		1		1		2	1	1		1			41
	97	94	78		2		5	3	1		3		1			187
	42	26					2	42			23		2			81
III_{79}...	46	39	47					10			1					65
	23	24	28				1				1					19
	18	12	17					16			4					1
	15	15	5				1	12			11					1
	32	39					4	44			56		1			
	62	64						46			63		1			
	34	34					1	33			40		1			
	1,431	1,608	1,101	...	6	...	37	687	7	2	631	0	11	0	0	938

TESTS OF CHANGED-OVER CLASSES

In a number of cases in which some members of an abnormal class changed over to become phenotypically members of a normal class; some of these apparently normal flies were tested under conditions favorable for the appearance in the next generation of abnormality.

These cases may be given. Two kinds of crosses are expected. In a few cases the normal will be found to be a true normal (single cross-overs) and give therefore only normal offspring when bred to normal (recessive). In other cases the expectation is for abnormal offspring, and where change of type has been extensive, these kinds will be in the majority.

In experiment II_{12} one gray red normal female when tested gave GRAb ♂ and ♀.

In experiment I_1 seven GRN ♀ were bred to BWN ♂. Four gave some abnormal offspring and three gave only normal offspring.

In experiment I_3 five GRN ♀ were tested. Three gave some abnormal, two gave only normal offspring.

In experiment II_5 seven GRN ♀ were tested. Four gave some abnormal offspring, and three gave only normal offspring.

In experiment III_1 one GRN ♀ tested gave some abnormals.

In III_2 one GRN ♀ bred to YRN ♂ gave some GRAb ♂♂ and ♀♀.

In III_5 some BRN ♂ were bred to their BRN sisters. All BR offspring were abnormal.

In III_{11} GRN ♀ paired to GRN ♂ gave GRAb ♂ and ♀.

In III_{20} GRN ♀ bred to GWN brothers gave GRAb ♀ and GWAb ♂ and ♀.

In III_{35} GRN ♂ to GRN ♂ gave GRAb ♂ and ♀.

In III_{35} GRN ♀ by GWN ♂ gave GRAb ♀ and GWAb ♂ and ♀.

In III_{42} GRN ♀ by GWN ♂ gave GRAb ♀ and GWAb ♂ and ♀.

In III_{54} GRN ♀ (17) by GWN ♂ (4) gave the same results as the last.

In III_{58} GRN ♀ × YWAb ♂ gave GRAb ♂ and ♀.

In III_{68} GRN ♀ to GWN ♂ gave GRAb ♂ and ♀ and YWAb ♂ and ♀.

In III_{79} GRN ♀ to GWN ♂ gave GRAb ♀ and GWAb ♂ and ♀ in three different tests.

In III_{90} one GRN ♂ (the only one present) when tested gave GRAb ♂ and ♀ and GWAb ♂ and ♀.

In III_{113} GRN ♀ to GWN ♂ gave GWAb ♂ and ♀ and GRAb ♀.

These results show without any question that in the great majority of cases the phenotypic normal class (when abnormality is expected) is in reality made up largely (entirely, except for cross-overs according to expectation) of genotypically abnormal individuals. Their abnormality is shown by suitable breeding tests such as those here recorded.

INFLUENCE OF THE FACTOR FOR BLACK ON THE REALIZATION OF THE ABNORMAL CONDITION

Some of the evidence seemed at times to indicate that flies heterozygous in black are less likely to show the abnormal abdomen, but even if this is true it is still uncertain whether this might not be due to other conditions than those caused directly by the heterozygosity for black. It might be that the black stock contained other factors that influence the cross. Moreover since the number of eggs laid by a given kind of female determines how many larvæ will appear in a given time, and since the relation of the larvæ to the food is an important factor in the results, it seemed hazardous to put any emphasis on such results.

In order that the heterozygous flies might be reared under conditions that the control showed were favorable for development of the abnormal condition in homozygous forms, some black, red-eyed normal females were mated to gray, white-eyed abnormal males. After the females were fertilized they were put into a new bottle with some of the stock white-eyed females (fertilized). Some of the daughters were red- and some white-eyed; all of the latter were very abnormal, but the red-eyed females (heterozygous) were all normal through five counts. At the fifth count the white-eyed males that had been abnormal up to this time became normal. The result is in accord with many similar observations; for as conditions

alter, the abnormal males first change to normal, then the heterozygous females, and lastly the homozygous females.

Several attempts were made to find out if, when the F_1 female, heterozygous for abnormal abdomen, is herself abnormal, her offspring are more likely to be abnormal than when she is normal. There is evidence everywhere throughout the tables to show that the condition of the mother has absolutely no effect on her offspring.

In December–January, 1914, the following experiments were made which are the converse, in one respect, of some of the preceding experiments since black abnormal females were used. The crosses are indicated below.

(1) Black, white, abnormal ♀ by gray, white, normal ♂.
(2) Gray, red, abnormal ♀ by gray, white, normal ♂.
(3) Black, white, abnormal ♀ by black, white, normal ♂.
(4) Gray, white, abnormal ♀ by black, white, normal ♂.

The F_1 females from (1) compared with (2) should show whether females heterozygous for black (and abnormal) are less abnormal than those pure for gray; provided, white and red eye make no difference in the development of abnormality. The F_1 female from (3) compared with (4) should reveal whether pure black heterozygous for abnormal are less abnormal than flies heterozygous for gray as well as abnormal.

The results need not be given in detail. It was found that the (F_1) daughters from (1) show the same degree of abnormality as those in (2). Hence heterozygosis in black need not have any influence on the realization of abnormality. The mothers were not, however, in the same bottles, but in different cultures kept as much alike as possible. To this extent the experiments are unsatisfactory. It was found that F_1 females from (3) were like those from (4), hence no evidence was found that the heterozygous type is more affected than the homozygous black. But here also the flies were reared in different bottles. In order to overcome this difference, some abnormal females that were heterozygous for black were bred to black normal males (both having white eyes).

The daughters were either heterozygous for black or pure black, likewise the sons. Hence, direct comparison could be made. The following protocol gives the results for four successive counts:

1.	Black ♀ quite abnormal.	Intermed. ♀ quite abnormal.
	Black ♂ quite abnormal.	Intermed. ♂ quite abnormal.
2.	Black ♀ quite abnormal.	Intermed. ♀ quite abnormal.
	Black ♂ quite abnormal.	Intermed. ♂ quite abnormal.
3.	Black ♀ fairly abnormal.	Intermed. ♀ fairly abnormal.
	Black ♂ very abnormal.	Intermed. ♂ very abnormal.
4.	Black ♀ none present.	Intermed. ♀ fairly abnormal.
	Black ♂ {one quite, one fairly abn.	Intermed. ♂ quite abnormal.

The evidence shows no difference between the extent of development of abnormality in the homozygous black and heterozygous black females and males.

In another way an attempt was made to get light on the same question. Red black females were mated to white abnormal males; and, simultaneously, red gray females were mated to white abnormal males. The females were later put into the same bottle and their offspring reared together. All the daughters for four counts were normal. At the fifth count an attempt was made to separate the two classes of daughters, which is possible, because the offspring heterozygous for black are darker than the grays. The heterozygous pairs were normal or slightly abnormal while the pure grays appeared a little more abnormal; but the difference is hardly to be relied upon, since the abnormality is less striking in the black flies.

To test this possibilty some of the preceding experiments were carried to F_2, when pure abnormal grays, intermediate and pure abnormal blacks appear. The most abnormal grays were no more abnormal than the most abnormal blacks, which so far as it goes shows that the homozygous black flies themselves may be as abnormal as the grays under the same conditions and with the same ancestry.

INFLUENCE OF THE FACTOR FOR YELLOW IN THE REALIZATION OF THE ABNORMAL CONDITION

Experiments similar to those with black were made with yellow. Yellow, white-eyed normal flies were bred to gray, white-eyed abnormal. In ten tests, the F_1 females were abnormal in eight cases, and normal in two. It is not apparent that the yellow factor has any decided influence on the results.

In order to compare the females heterozygous in black and in yellow, and others also homozygous in both, the four following tests were made. By utilizing the red and the white eye colors it was possible to distinguish between the different classes of females. Previous experiments, described above, had made it highly probable, that no effects are produced by red and by white, but by making reciprocal crosses here this possible effect was more certainly eliminated. In all cases the females were mated separately for a few days to gray, white-eyed abnormal males to better ensure fertilization and were then brought together in one bottle.

(1)

{YWN ♀ by GWAb ♂.
{BRN ♀ by GWAb ♂.

1. {GR ♀ Fairly Ab.
   {GW ♀ Fairly Ab.
2. {GR ♀ Like last.
   {GW ♀ Like last.
3. {GR ♀ Like last.
   {GW ♀ Like last.

(2)

{BWN ♀ by GWAb ♂.
{YRN ♀ by GWAb ♂.

1. { 3 GRN ♀.
   { 1 GWN ♀.
2. {12 GR slightly Ab ♀.
   { 3 GW slightly more Ab ♀.
3. { 1 GRN ♀.
   { 8 GW slightly Ab ♀.
4. { 4 GRN ♀.
   { 8 GWN ♀.
5. { 1 GRN ♀.
   {10 GWN ♀.
6. { 2 GRN ♀.
   {14 GWN ♀.

In (2) the females heterozygous in yellow were slightly more abnormal than those heterozygous in black or at least they gave such an impression.

(3)

BRN ♀ by GWAb ♂.
GWN ♀ by GWAb ♂.

1. GR slightly Ab ♀.

2. GRN or nearly N ♀.
GW fairly Ab ♀.

3. 5 GRN and 4 GR quite Ab ♀.
GW ♀ quite to fairly Ab.

4. 13 GRN ♀.
16 GW quite to slightly Ab ♀.

5. 15 GRN ♀.
9 GW fairly Ab to N ♀.

6. 6 GRN ♀.
10 GWN ♀.

(4)

BWN ♀ by GWAb ♂.
GRN ♀ by GWAb ♂.

1. 5 GR slightly Ab ♀.
2 GW nearly N ♀.

2. 14 GR fairly to slightly Ab ♀.
6 GW nearly N ♀.

3. GR slightly Ab to N ♀.

4. 28 GRN ♀.
3 GWN ♀.

5. 17 GRN ♀.
2 GWN ♀.

6. 6 GRN ♀.

In (3) the females heterozygous in black were slightly more normal than the grays. In (4) there is hardly any difference, but so far as difference is noticeable the heterozygous type (GW) is again more nearly normal. This difference was even more apparent in a second culture from the same parents.

THE RELATIVE INFLUENCE OF THE EGG AND OF THE SPERM ON THE CONDITION OF THE HETEROZYGOTE

At the time when the F_1 generation began to hatch the extent of the abnormality in the females was noted. This was at the time when the flies were taken out to become the parents of the F_2 generation. The terms used were necessarily somewhat vague, but give a fairly accurate idea of the condition of the cultures as a whole. If most of the flies were distinctly abnormal this was indicated by Ab to N, if more of the flies were normal or nearly so but some were abnormal this was indicated by N to Ab. If the flies were normal in appearance this was indicated by N. The results for many of the cases recorded in the preceding tables are brought together in the next table.

The results are far from uniform, as was to be expected, but in most cases it will be noted that when the female was normal and the male abnormal, the daughters were frequently normal or nearly so, while in the reciprocal cross the tendency was in the opposite direction, *i. e.*, the

daughters were more likely to be abnormal. These records were made at a time when no suspicion of such a relation was present in my mind. If these observations are to be trusted they mean that when abnormality comes

	Abn.	Abn. to N.	N. to Abn.	N.
GWN♀ YRAb♂			2	3
YRAb♀ GWN♂				
YRN♂ GWAB♂	3	2	10	10
GWAb♀ YRN♂	3			
GRN♀ YWAb♂			1	7
YWAb♀ GRN♂				2 (few)
YWN♀ GRAb♂	14	2	6	1
GRAb♀ YWN♂			1	1
GWN♀ BRAb♂	3		3	4
YWN♀ BRAb♂				2
GWN♀ GRAb♂	4	1		1
GRAb♀ GWN♂	8			

in with the egg the heterozygous female is more likely to show abnormality than when the abnormality comes in with the sperm. Conversely the result may be stated in this way—when normality comes in from the egg the daughters are more likely to be normal than when the normality comes in from the sperm. In other words, we might extend this conclusion and state that the cytoplasm of the egg has an influence on the soma of the individual which arises from it, or the cytoplasm plus the nucleus of the egg has more influence on the next generation than the nucleus of the sperm.

When this possibility was realized it was evident that some of the experiments must be repeated under condi-

tions where a more exact comparison between a cross and its reciprocal could be made. In the autumn of 1913 I went over the ground again with this object in view. It was found that the F_1 females heterozygous for abnormality are just as likely to be abnormal when their abnormal factor comes from the father as when it comes from the mother. The extent to which the abnormality is realized depends on the condition of the food. This in turn will depend in part not only on its amount but to what extent it is worked over by the larva which again depends, in large part, on the number of eggs laid by the female. To this extent and only in this sense does the condition of the mother affect the condition of her daughters. If the females lay too many eggs for the amount of food that is present, crowding results and the daughters show abnormality to a less degree than when fewer eggs are laid (that hatch) and little competition takes place. Now the normal female is more likely to lay more fertile eggs than the abnormal female. Hence other things being equal the heterozygous daughter of a normal mother is more likely to be normal than the heterozygous daughters of an abnormal female (which are therefore again more like their mother—very abnormal in this case, because the former mother is more likely to lay more eggs than the latter). The relation between the two cases is therefore not owing to the egg transmitting abnormality to the daughters better than the sperm, but to the number of eggs likely to be laid by the mother in question.

In order to examine further whether when abnormality comes in with the egg it is more likely to be shown in the F_1 heterozygote, a number of parallel experiments were made, of which the following are samples:

Gray Red Abn. ♀ by Wild ♂.	Gray Red Abn. ♂ by Wild ♀.
(1) Very abn. ♂ and ♀.	(1) Fairly abn. ♀.
(2) Normal (a few slightly abnormal).	(2) Most fairly, a few very abn.
	(3) Most fairly, a few very abn.
(3) Normal (a few slightly abnormal).	(4) Slightly abn.
	(5) Slightly abn.
(4) Normal (a few slightly abnormal).	(6) N ♀ (40) 1 slight abn. ♀.
	(7) N ♀.

While it is true in the first count above that when abnormality entered through the egg there was greater abnormality in the offspring, yet this is offset by the counter evidence in this set that the change to the usual phenotype took place sooner in this set than in the others. This point will be taken up again in connection with other data.

In order to compare, under changing conditions, heterozygous and homozygous females, some white abnormal females were mated to red abnormal males, and, independently, some other white abnormal females were mated to red normal males. After several days both kinds of females were separated from their respective males and put together into a single new bottle. All of the daughters had red eyes. In the first count two types of females could readily be distinguished. Some were quite abnormal, others were slightly abnormal or normal. In the second count (next day) again two types appeared, one quite abnormal and the other slightly abnormal females. In the third count some females were fairly abnormal, the rest normal and this held for the fourth count. The result leaves little doubt that under these conditions, the homozygous were abnormal and the heterozygous less abnormal or quite normal.

In order to see if the factors for red and for white affect the condition of the zygote, homozygous for abnormal; white abnormal females were mated to red abnormal males, and, separately, other white abnormal females to white abnormal males. After several days the females were put together in a new bottle and the males removed. Through five consecutive counts the red and the white daughters were alike, at first quite abnormal, later nearly normal. Red and white abnormal females therefore behave alike.

PRESENCE AND ABSENCE

It is not without interest to examine the bearing of these results from the point of view of the "presence

and absence" hypothesis, even although I myself prefer a more non-committal form of factorial interpretation than that offered by the "presence and absence" theory.

The abnormal male (Ab) has one dose of abnormality and the degree of his abnormality is the same as that of the female (Ab, Ab) with two doses. But the heterozygous female, AbN, has only one dose (or factor) for abnormality. The degree of abnormality that she shows is very variable; she is less abnormal on an average, than the abnormal male.

Which condition is to be interpreted as absence—the real absence of one Ab in the male, or the absence of one Ab in the other (normal) chromosome of the female? A moment's thought will show, however, that nothing of any value can come from a discussion of this question, because the heterozygous female (AbN) differs from the male not simply by the factor N, but by a whole chromosome including amongst other factors a factor which in duplex produces a female. Moreover, an advocate of presence and absence might maintain that the relation of a dominant to the normal allelomorph is not the same as the relation of a normal allelomorph to a recessive for it is the latter that is "absent." In other words, he might conceivably accept the hypothesis of absence for a recessive, but reject it for a dominant mutation.

I have pointed out elsewhere that it seems to me unwarrantable to interpret the absence of a character to mean necessarily an absence of a factor in the germ plasm.[5] Yet this literal interpretation of the presence and absence hypothesis has often been made. If the linear arrangement of factors in the chromosomes be admitted as a plausible hypothesis the absence of a factor in this literal sense would mean a hole in the chromosome, and a corresponding displacement of the linear sequence of factors. The evidence does not support this hypothesis. On the other hand, if the locus of a factor be con-

[5] Although of course a changed factor might cause the failure of some substance to develop that is necessary for a given reaction.

ceived as a particular chemical body at a given level in the chromosome then any change in this body would be expected to affect one, or more, or even, at times, all characters of the complex that gives rise to the body character or characters. The particular change might involve no more than a rearrangement of the materials of the locus or the addition of a chemical element (or compound) or the loss of a chemical element (or compound) —any one of these changes might lead to the loss of a character in the soma. As to what happens in the locus we can form no idea, and so far as the mechanism of heredity is concerned it is a matter of no immediate importance. If, however, any one finds a greater satisfaction in the view that a loss of something from the locus (an atom or a molecule) leads to a recessive character, there is not the slightest objection to his doing so, unless by loss he means the loss of the entire locus. He may do this if he rejects the linear arrangement of different material in the chromosomes, but if he accepts the latter view the assumption of a literal absence involves him in unnecessary difficulties. It is not as generally understood as it should be that the facts which the presence and absence theory was constructed to account for do not require the assumption that the absence of a character means the absence of a factor in the germ-plasm. It is entirely gratuitous to involve the theory of Mendelian heredity in such an interpretation which adds nothing to the theory and by bringing in a new hypothesis may involve the Mendelian theory in further difficulties. An example may make this clear. It is known that when a chocolate mouse is bred to gray and the F_1 grays that result are bred together there appear in F_2 grays (9), cinnamons (3), blacks (3) and chocolate (1). Gray was written GBCh and chocolate gbC, which gave in F_2 GBCh (9) GbCh (3) gBCh (3) and gbCh (1). The occurrence of the black class of gBCh is accounted for through recombination. But the same end is accomplished if we

suppose that a factor in the wild or agouti mouse mutated so that the recessive black was produced as a result of the activity of the new gene. Then bl = black, and Bl = gray with respect to black. Likewise cinnamon agouti may be represented by ci, and gray, with respect to cinnamon, by Ci. Chocolate is then the double recessive blci and the symbol Ch for "chocolate" becomes superfluous. All the experimental results may be explained on this basis.

It is not necessary to try to state what kind of a change in the germ-plasm led to these two mutations. The factorial hypothesis should be entirely non-committal as to the kind of change that took place, for we can know nothing about the nature of the change, yet the results are predictable as well on one view as on the other.

There is another way to interpret a dominant factor like this one that gives abnormality, namely, that there is present in the normal fly a factor that restricts the yellow of the abdomen to the bands. When this restrictor, ab, changes (Ab) the yellow is dispersed over the abdomen and the black bands fail in part or entirely to appear. The new factor, acting with the rest of the cell, gives abnormality, just as the normal restrictor or inhibitor (ab) acting with the rest of the cell gives normality or banding. The interpretation is non-committal in regard to the nature of the change, which is an advantage in the direction of simplification. In contrast to this view, a different interpretation of the meaning of a restrictor might be entertained on the presence and absence view. It might be said that a restrictor factor has been "lost" from the normal fly, which failing to restrict the color has given rise to abnormality. The first objection to this hypothesis is that it postulates (as above) the nature of the change in the germ plasm, because it says something has been lost. The second objection is that the facts show that a restrictor has not been lost *sensu strictu because there is a wide range of variation in regard to*

the loss of banding and in certain environments there is a return to the normal banding to the extent that the fly can not be distinguished somatically from a normal banded fly.[6] My contention is that since we know nothing of the nature of the change in the germ-plasm that leads to the appearance of a new or the loss of an old character, any assumption that is based on the nature of that change involves the Mendelian interpretation in unnecessary implications. We need only assume that some change has occurred, as the result indicates; my formulas give the same results as do those of presence and absence and serve the purpose of briefly indicating a change, the machinery involved, and the necessary consequences.

OTHER TYPES OF ABNORMAL ABDOMEN

Irregularities in the arrangement of the rings of the abdomen are not uncommon in *Drosophila*. Sometimes they appear to have been caused by injury to the larvæ or pupæ, but still other abnormalities are inherited in the sense that they occur in certain stocks in more or less definite percentages. Several times I have bred abnormal types: some of them have failed to reappear; others have reappeared in a certain percentage of cases. Two stocks of the latter kind may be referred to here. My main purpose in describing them is to anticipate the possible confusion that might arise if some one finding these or similar ones should suppose them to be the same types as those described as abnormal abdomen in this paper.

The six drawings in Fig. 2, *a–f*, represent some of the characteristic types of a certain stock. The failure of the third abdominal ring to extend across the middle line, as

[6] It is not an objection to this hypothesis that an absence (loss of restrictor) appears to dominate presence. This interpretation rests on a complete misunderstanding of the nature of the factorial hypothesis; for, absence here means only that the rest of the cell fails to produce banding when a certain factor is lost, or, when as in the female, one of the inhibitors is lost.

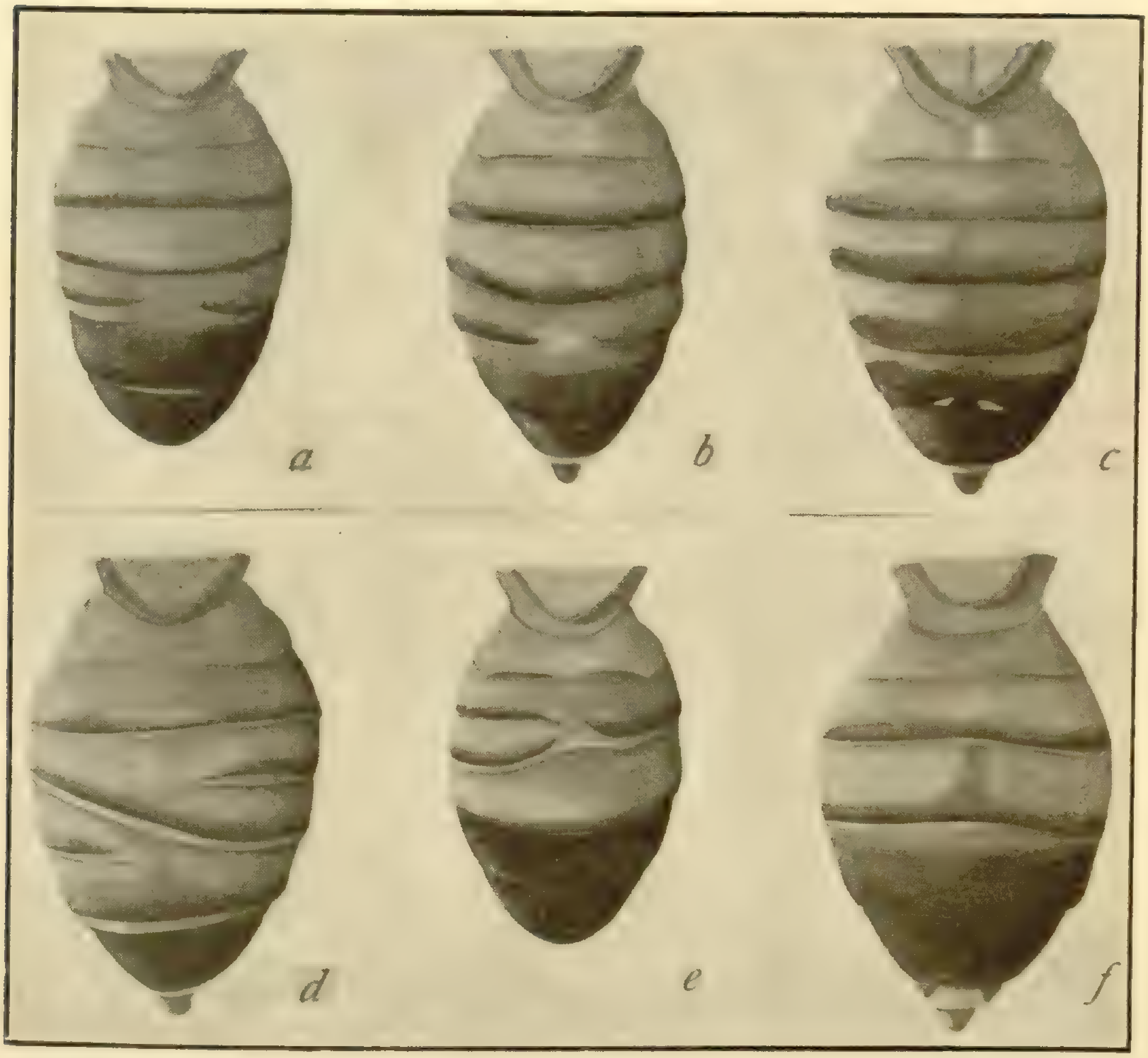

FIG. 2.

seen in the first two figures, is the more usual form of abnormality in this stock; but modifications of other rings shown in the other figures are probably due to the same cause or causes. Two consecutive rings may form a spiral as shown in *c* or half of a ring may be absent as in *e*, or an entire ring may be lacking as in *f*. Individuals with abnormalities like those shown in the figures were bred to each other usually three or four together. Their progeny was examined and the normal and the abnormal types recorded. The latter were again used to breed from for three or four generations. As no increase in the proportion of abnormal offspring appeared, the breeding was abandoned. The results given below are in the order in which they were obtained without regard to the generation in which they appeared.

In these counts there were normal to abnormal flies both of whose parents were abnormal. Since the normals also throw some abnormals it is probable that there is here a case of multiple factors like that of beaded and truncate. Special tests will therefore be necessary to work out the case.

N	Ab	N	Ab	N	Ab	N	Ab
85	7	43	13	40	0	73	0
6	1	13	2	18	1		
28	5	33	2	52	7	32	5
32	10	20	2	15	5	105	3
15	3	13	4	26	1	37	10
40	6	24	7	23	2		
31	6						

The abnormal abdomens shown in Fig. 3, *a–f*, are from another stock, discovered by Mr. Bridges. While some of the types are not unlike those of the last series, they are more extreme and there can be no doubt but that the two stocks have a different composition.

In the last drawing the entire fly is figured (the one wing present has been cut off at the base), the upper half of the thorax is absent. This same condition appears in rather high proportions in certain other stocks, notably in vestigial stocks. Even both sides of the thorax may be absent so that the head rests above on the abdomen. Although I have tried a number of times to obtain pure stocks of this thoracic abnormality, I have never succeeded in getting a stock that did not throw a high percentage of normal individuals.

This type of abnormal abdomen appeared in a cross between a cream male and an eosin female as a single female, Fig. 1, *a*, which had only three instead of five bands in the abdomen. She was mated to one of her brothers, and produced offspring all of which as far as known had normal bands. A pair of these offspring gave in the next generation abnormal bands in about half of the flies. The abnormal band acted as a recessive. In subsequent generations the character behaved in an irreg-

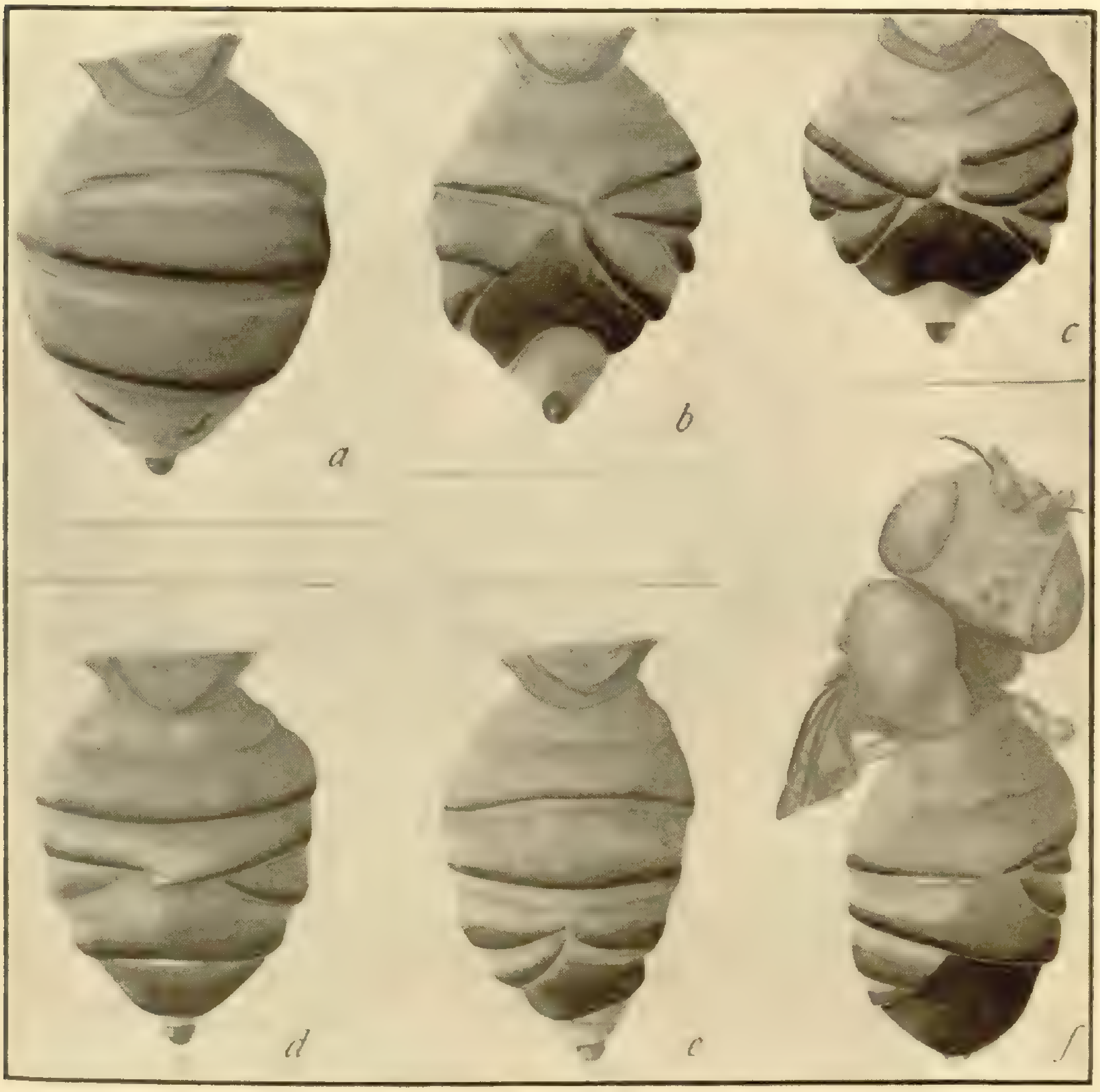

Fig. 3.

ular manner though no serious attempt was made to discover the cause of the irregularity. A stock of cream eye color was made up from this strain and selection against the abnormal was carried out in a rough way for several generations, but this selection failed to eliminate the abnormal condition, and a recent examination of the stock showed that for a year the abnormal abdomen had maintained itself and was still present in about half of the flies.

A male was again crossed to a wild type female and gave normal F_1 flies. In F_2 there were 128 red normal females, 29 red normal males, and 28 eosin normal males. No abnormals appeared. Crossed to eosin the F_1 were

PARENTS: AbE BY NE

F_1: NE

EN ♀	EN ♂	EAb ♀	EAb ♂
78	56	1	1
13	14	0	2
46	40	0	2
6	7	0	0
35	32	0	1
39	46	7	1
217	195	8	7

all normal; these inbred gave in F_2 the classes given above: Two eosin females heterozygous for white were crossed each to an abnormal male. The normal F_1 daughters were bred to those sons that had white eyes, and gave the following kinds of offspring:

PARENTS: E–WN ♀ BY EAb ♂

F_1: EN ♀ (BY WN ♂)

EN ♀	EN ♂	EAb ♀	EAb ♂	WN ♀	WN ♂	WAb ♀	WAb ♂
18	26	1	1	20	15	1	1
34	28	1	2	18	25	2	1
52	54	2	3	38	40	3	2

Abnormal males were bred to eosin females and gave, as before, normal F_1 sons and daughters. Some of the daughters were backcrossed to eosin cream abnormal males and gave the following results:

PARENTS: AbE BY NE

F_1: NE (BY AbE ♂)

EN ♀	EN ♂	EAb ♀	EAb ♂
35	32	0	1
21	4	1	2
48	40	0	1
72	72	8	6
176	149	9	10

These tables show that the abnormal condition rarely appears in F_2. Its realization must be due therefore either

to multiple factors or to environmental effects. That the former rather than the latter is the main explanation is shown in the frequency with which the abnormal flies appear in the inbred stock (where the conditions are the same as in the experiments) and the rarity with which the character appears when the stock is outcrossed.

THE NON-INHERITANCE OF AN ACQUIRED CHARACTER

The acquirement of a new character by a pure stock implies by definition the capacity of this stock to respond to the imposed conditions. Conversely if an animal does not acquire a new character in a changed environment it does not come within the scope of the definition of an acquired character, and even should its offspring show new characters as a result of the new environment in which the parents have been placed the result is still excluded by definition from being a case of the inheritance of an acquired character. At least this is my understanding of the use of the term and the way in which I shall use it in the following statement.

The mutant stock of abnormal abdomen offers an exceptional opportunity to examine the possible influence of an acquired character on the offspring. As the experiments have shown this stock is very susceptible to environmental influence, and the effects produced profoundly affect the structure of the organism. Moreover it is possible to carry the stock through several generations in either of the phenotypic conditions, and then, at will, to cause the other type to appear at once in its completest form, by regulating the external conditions in which the young are reared.

No better material could be found for studying the possible influence of the environment through its effects on the soma of the individual. *The evidence shows in the clearest manner that the condition of the parent, whether normal or abnormal in type, has no effect on the character of the offspring.* The evidence is so clear and so positive that it seems unnecessary to elaborate the point.

THE NON-CONTAMINATION OF GENES

Recently the question of the possible contamination of genes (or factors) has been under discussion. Were such contamination possible one might expect to find some evidence of it in a case like this one of abnormal abdomen, if one is justified, at all, in drawing inferences from the nature of the character to the nature of the gene that stands for that character. I do not myself think that there is the slightest justification in drawing such conclusions, but let us assume for the moment that such an inference is justifiable in order to examine the bearing of the evidence furnished by this mutant type.

The heterozygous female carries a factor for normal and one for abnormal. She herself may be either normal or abnormal according to the environment in which she was reared. It might be supposed, since she is abnormal, that her normal gene might be more predisposed to contamination by the abnormal gene. The evidence shows that this does not occur; for, by means of the linkage we can identify the normal flies that should carry the normal, or the abnormal genes, and we find that the results conform completely to expectation; *i. e.*, they are in full accord with all other linkage results where there is no reason to suppose that contamination takes place.

Conversely it might be supposed that if the heterozygous female were normal in type her abnormal gene might be predisposed to contamination by the normal gene, but again the evidence contradicts the assumption.

If, on the other hand, it is *not* supposed that the phenotypic condition of the female has any part in bringing about contamination (or in serving as an indicator, that conditions are favorable for contamination) but that contamination is due merely to juxtaposition of genes in the same cell, then in refutation of the contamination of genes I may cite the evidence cited above, where in several successive generations the breeding took place from heterozygous females bred to recessive males and the gametic

ratios were the same in the late as in the earlier generations.

Lastly the tests made of individuals that were phenotypically normal, but genetically abnormal, showed in all cases the validity of the genetic evidence, which would not have been the case if the apparent exceptions had been due to contamination of the genes. I may also cite the two peculiar matings, *B* and *C*, recorded on page 402, where an expected class did not appear. It might have appeared that here actual contamination had occurred. In reality, the result turned out to be due to a lethal factor. Our study of these lethals, that give verifiable results, fully under control, made it possible to interpret this case that otherwise would have been inexplicable, and might have been cited in favor of the view of contamination of genes. Taken all together the results obtained with this mutant type make out a strong case against the supposition that genes become contaminated through juxtaposition. I shall not discuss here, therefore, the unpragmatic character of such a supposition, but rest the case on the evidence from the experiments.

ON A CRITERION OF SUBSTRATUM HOMOGENEITY (OR HETEROGENEITY) IN FIELD EXPERIMENTS

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I. INTRODUCTORY REMARKS

EVERY ONE who has had practical experience in variety or fertilizer tests or in any other experiments involving the comparison of field plots must have been impressed by the great difficulty of securing tracts with uniform soil for their cultures.

A careful examination of the agricultural literature bearing on the question of variety tests will reveal many cases in which the experimenters have noted the difficulty of securing a uniform substratum, or in which there is internal evidence for the influence of substratum heterogeneity upon the result.

For example, in 1894–1895 tests of varieties of wheat were made on 77 plots at the University of Illinois.[1] As a check on the other strains, the variety known as Valley was sown on nine different plots "well distributed over the area sown."

> . . . the yields of this variety varied from 11.7 bushels to 24.1 bushels, an average of 19 bushels which is remarkably close to the average of all the varieties. It is again remarkable that but eight yields were above the highest of the Valley, and but three below the lowest of the same variety, . . .

The only reasonable explanations that can be given for such results are either (*a*) that the plots were so small that the results are due purely and simply to the errors of random sampling, or (*b*) that the wide divergences in the

[1] *Bull. Univ. Ill. Agr. Exp. Sta.*, 41, 1896.

results for the individual variety are due to substratum heterogeneity.

In either case, the results secured are obviously worthless as indicating differences in the value of the individual varieties.

Seventeen years ago, Larsen[2] reached the conclusion that the results of experimental tests were much more exact when a given area is divided into a large number of small plots upon which the tests are made than when it is divided into a few larger plots.

Hall[3] has laid great emphasis upon irregularities of experimental fields. Mercer and Hall in their interesting paper on "The Experimental Error of Field Trials"[4] discuss at considerable length various phases of the influence of soil heterogeneity upon field results. In an appendix to their paper, Student[5] takes up the problem of the method of arranging plots so as to utilize to the best advantage a given area of land in testing two varieties.[6]

The influence of substratum heterogeneity is also readily seen in Montgomery's interesting experimental data for wheat.[7]

Indeed, it is quite possible that without special precautions irregularities in the substratum may have greater influence upon the numerical results of an experiment than the factors which the investigator is seeking to compare. Elsewhere[8] I have shown that the differentiation

[2] Larsen, B. R., "Andra nordska Landbrakskongressen i Stockholm," 1897, I, p. 72; *fide* G. Holtermark and B. R. Larsen, *Lanwirtschaftl. Versuch-Stationen*, 65, 1, 1907.

[3] Hall, A. D., "The Experimental Error of Field Trials," *Journ. Board Agr. Great Britain*, 16, 365–370, 1909.

[4] *Journ. Agr. Sci.*, 4, 107–127, 1911.

[5] Student, *Journ. Agr. Sci.*, 4, 128–132, 1911.

[6] For several years, I have in careful tests labelled each seed individually and scattered them at random over the field to eliminate the influence of soil heterogeneity

[7] Montgomery, E. G., "Variation in Yield and Method of Arranging Plots to Secure Comparative Results," *Ann. Rep. Neb. Agr. Sta.*, 25, 164–180, 1912.

[8] Harris, J. Arthur, "An Illustration of the Influence of Substratum Heterogeneity upon Experimental Results," *Science*, N. S., 38, 345–346. 1913.

in an apparently uniform garden plot may be sufficient to mask entirely the influence of the weight of the seed (*Phaseolus vulgaris*) planted upon the size of the plant (as measured by the number of pods) produced. It is very probable that certain pure-line experiments and conclusions are entirely invalidated by the fact that the influence of irregularities in the substratum were not sufficiently guarded against.[9]

Several authors have tried to obtain some measure of, or some corrective term for, substratum heterogeneity. For example, Mercer and Hall (*loc. cit.*) have plotted the yields across the field in both directions. Such methods, however, give but a very imperfect idea of irregularities in the soil. Heterogeneity is perhaps more likely to occur as a spotting of the field than as a relatively uniform change from one side to the other. This is clearly indicated in the diagrams published by Montgomery. The mere plotting of yields in any line across the field can not adequately take into account such irregularities. Furthermore, some quantitative measure (and some probable error of this measure) of the amount of irregularity, not merely a demonstration of its existence, is required.

Some generally applicable measure of the degree of homogeneity of the soil of a field (as shown by actual capacity for crop production) seems highly desirable. Such a criterion should be universally applicable, comparable from species to species, character to character or experiment to experiment, and easy to calculate.

I believe we may proceed as follows. Suppose a field divided into N small plots all planted to the same variety of plants. Let p be the yield of an individual plot. The variability of p may be due purely and simply to chance, since the individuals of any variety are variable and the size of the plots is small, or it may be due in part to differentiation in the substratum. If the irregularities in the experimental field are so large as to influence the yield of

[9] See "The Distribution of Pure Line Means," AMER. NAT., 45, 686–700, 1911.

areas larger than single plots[10] they will tend to bring about a similarity of adjoining plots, some groups tending to yield higher than the average, others lower.

Now let the yields of these units be grouped into m larger plots, C_p, each of n contiguous ultimate units, p. The correlation between the p's of the same combination plot, C_p, will furnish a measure (on the scale of 0 to 1) of the differentiation of the substratum as expressed in capacity for crop production. If this correlation be sensibly 0, the irregularities of the field are not so great as to influence in the same direction the yields of neighboring small plots. As substratum heterogeneity becomes greater, the correlation will also increase. The size of the coefficient obtained will depend somewhat upon the nature of the characters measured, somewhat upon the species grown, and somewhat upon the size of the ultimate and combination plots. A knowledge of the values of the correlation to be expected must be determined empirically.

Fortunately, very simple formulæ are now available for calculating such coefficients.[11]

Let S indicate a summation for all the ultimate or combination plots of the field under consideration, as may be indicated by the capital C_p or lower case p. Then in our present notation which is as much simplified as possible for the special purposes of this discussion

$$r_{p_1p_2} = \frac{\{[S(C_p^2) - S(p^2)]/m[n(n-1)]\} - \bar{p}^2}{\sigma_p^2}$$

where $\bar{P}$ is the average yield of the ultimate plots and σ_p their variability, and n is constant throughout the m combination plots.[12]

[10] Irregularities of soil influencing the plants of only a single small plot may in most work be left out of account, since they are of the kind to which differences between individual plants are to a considerable extent due, and are common to all the plots of a field.

[11] Harris, J. Arthur, "On the Calculation of Intra-class and Inter-class Coefficients of Correlation from Class Moments when the Number of Possible Combinations is Large," *Biometrika*, 9, 446–472, 1913.

[12] For the benefit of those who are frightened by formulæ, it may be paraphrased as follows: One merely adds together the yields of a chosen

Ultimately, I hope to present experimental data of my own bearing on this problem. For the present, the method is admirably illustrated by a number of published records.

II. ILLUSTRATIONS OF METHOD

A. Cases in which the Combination Plots are Equal in Size

Illustration 1. *Influence of substratum heterogeneity on yield of experimental plots of mangolds.*

TABLE I

YIELD OF COMBINATION PLOTS FOR MANGOLDS, OBTAINED BY COMBINING THE ENTRIES OF MAP A BY FOURS AS INDICATED BY THE HEAVIER LINES

1,209 172	1,175 183	1,215 171	1,239 175	1,276 205
1,250 185	1,321 191	1,274 187	1,293 184	1,310 207
1,204 159	1,333 188	1,268 172	1,290 185	1,268 200
1,300 172	1,272 177	1,222 167	1,272 173	1,388 215
1,385 193	1,375 194	1,314 193	1,260 180	1,373 219
1,380 204	1,387 202	1,309 177	1,314 188	1,380 229
1,320 180	1,295 188	1,304 187	1,332 194	1,397 226
1,331 183	1,264 183	1,310 188	1,325 183	1,337 203
1,404 194	1,325 190	1,334 190	1,335 192	1,312 211
1,418 193	1,373 196	1,339 189	1,403 198	1,401 226

number of contiguous p plots to form a number m of C_p plots. The sum of the squares of p is subtracted from the sum of the squares of C_p and the result divided by $m[n(n-1)]$ where n is the number of ultimate plots in each of the m combination plots. The quotient is reduced by subtracting the square of the mean yields of the ultimate plots, $\bar{p}$, and the remainder divided by the square of the standard deviation of yields of ultimate plots, σ_p^2. The quotient is the correlation between the yields of the ultimate units, p, of the same combination plot, C_p,—the measure of heterogeneity required. Thus the calculation of the criterion is very simple indeed.

NW	1	2	3	4	5	6	7	8	9	10
1	310 42	302 46	288 41	325 48	321 45	291 41	306 41	306 45	306 48	330 57
2	290 41	307 43	267 40	295 54	308 45	295 40	317 44	310 45	316 48	324 52
3	322 49	309 45	322 43	324 49	330 52	286 40	300 46	325 47	302 47	298 49
4	309 44	310 47	324 46	351 53	342 51	316 44	324 44	344 47	341 51	369 60
5	278 38	320 42	335 46	350 51	342 49	309 40	310 45	322 48	329 50	300 52
6	302 37	304 42	310 45	338 46	316 43	301 40	328 44	330 48	325 46	314 52
7	306 41	318 42	302 39	332 50	299 43	277 37	331 44	322 46	384 61	339 52
8	333 45	343 44	318 44	320 44	335 47	311 40	296 38	323 45	327 49	338 53
9	339 44	336 46	324 46	347 47	331 51	313 42	312 48	300 41	346 52	343 57
10	360 51	350 52	354 51	350 50	348 54	322 46	325 43	323 48	335 50	349 60
11	346 51	362 55	372 51	349 54	343 46	308 40	297 43	328 49	352 56	309 55
12	327 47	345 51	331 48	335 49	342 49	316 42	339 47	350 49	365 54	354 64
13	310 45	364 50	300 46	337 45	321 46	329 46	341 51	349 49	363 58	347 59
14	317 41	329 44	321 45	337 52	340 50	314 45	321 48	321 46	346 52	341 57
15	323 44	326 44	290 41	328 48	348 49	325 45	358 48	332 44	349 51	335 56
16	353 49	329 46	311 47	335 47	331 46	306 48	318 47	317 44	332 46	321 50
17	357 52	348 46	301 44	335 47	340 51	336 45	327 46	330 50	343 54	317 54
18	362 46	337 50	339 47	350 52	328 47	330 47	343 49	335 47	326 47	326 56
19	349 46	365 52	359 48	339 47	340 48	332 49	356 50	336 48	338 51	316 56
20	352 49	352 46	340 50	335 51	332 44	335 48	356 50	355 50	371 52	376 67

MAP A. Pounds per Plot of Roots and Leaves of Mangolds. Data of Mercer and Hall.

Map A represents the Rothamsted field of mangolds grown by Mercer and Hall (*loc. cit.*). The upper entries are for pounds of roots, the lower for pounds of leaves.

I now reduce the 200 areas to 50 by combining the adjoining plots by fours, as indicated by the heavier lines on the map. Thus for leaves the Southwest combination plot, C_p, is $67 + 52 + 56 + 51 = 226$. Table I gives the result.

This gives for roots:

$$S(p) = 65715, \quad S(p^2) = 21674871, \quad N = 200,$$
$$\bar{p} = 328.575, \quad \sigma_p{}^2 = 412.824,$$[13]
$$S(C_p{}^2) = 86537439, \quad m[n(n-1)] = 50 \times 4 \times 3 = 600,$$
$$[S(C_p{}^2) - S(p^2)]/m[n(n-1)] = 108104.280,$$

and

$$r_{p_1p_2} = \frac{108104.280 - (328.575)^2}{412.824} = .346 \pm .042.$$[14]

The results for yield of leaves are

$$S(p) = 9541, \quad S(p^2) = 45941, \quad N = 200,$$
$$\bar{p} = 47.705, \quad \sigma_p{}^2 = 23.938,$$
$$S(C_p{}^2) = 1832095, \quad m[n(n-1)] = 50 \times 4 \times 3 = 600,$$
$$[S(C_p{}^2) - S(p^2)]/m[n(n-1)] = 2286.923,$$

whence

$$r_{p_1p_2} = \frac{2286.923 - (47.705)^2}{23.938} = .466 \pm .037.$$

Illustration 2. *Influence of Substratum Heterogeneity upon the Yield of Straw and Grain in Experimental Plots of Wheat.*

[13] The standard deviation is most conveniently calculated in cases like the present, in which one requires the summed squares of actual values for other purposes from

$$\sigma_p{}^2 = \Sigma(p^2)/N - [\Sigma(p)/N]^2.$$

[14] The probable errors have in all cases been calculated upon the actual, not the weighted, number of ultimate plots as N.

The wheat field of Mercer and Hall is divided into $25 \times 20 = 500$ plots, Map B. Combining the plots by fives from east to west and by fours from north to south, I have condensed this into $5 \times 5 = 25$ C_p plots, each of 20 ultimate plots as shown in Table II.

TABLE II

Yields of Combination Plots of Rothamsted Wheat, 4×5 Grouping. Original Areas Separated by Double Lines in Map B

82.89 139.36	83.05 132.41	78.63 122.84	78.76 120.53	74.70 114.58
78.15 130.60	84.34 140.31	75.61 120.11	80.32 119.27	74.87 112.21
79.80 133.31	84.70 149.58	74.94 125.27	81.50 133.28	77.34 120.09
84.36 142.79	82.42 147.74	73.60 131.80	71.35 121.18	75.81 122.02
85.19 147.95	84.56 146.78	82.25 138.42	68.52 120.09	76.69 124.88

Summing the actual yields and the squares of yields for the ultimate plots and the squares for the combination plots, I find the following values:

For wheat grain

$$S(p) = 1974.32, \quad S(p^2) = 7900.6790, \quad N = 500,$$

$$\bar{p} = 3.949, \quad \sigma_p^2 = .209600,$$

$$S(C_p^2) = 156419.3106, \quad m[n(n-1)] = 25 \times 20 \times 19 = 9{,}500,$$

$$[S(C_p^2) - S(p^2)]/m[n(n-1)] = 15.633540,$$

which leads to

$$r_{p_1p_2} = \frac{15.633540 - (3.949)^2}{.209600} = .186 \pm .029.$$

For wheat straw

$$S(p) = 3257.40, \quad S(p^2) = 21623.9802, \quad N = 500,$$

$$\bar{p} = 6.515, \quad \sigma_p^2 = .805341,$$

$$S(C_p^2 = 427479.9920, \quad m[n(n-1)] = 9500,$$

$$[S(C_p^2) - S(p^2)]/m[n(n-1)] = 42.721685,$$

NW	1	2	3	4	5	6	7	8	9	10	11	12
1	3.63 6.37	4.15 6.85	4.06 7.19	5.13 7.99	3.04 4.71	4.48 6.08	4.75 7.31	4.04 6.08	4.14 6.98	4.00 5.87	4.37 6.75	4.02 6.10
2	4.07 6.24	4.21 7.29	4.15 7.41	4.64 7.80	4.03 6.34	3.74 6.63	4.56 7.88	4.27 6.35	4.03 6.91	4.50 6.50	3.97 6.09	4.19 6.43
3	4.51 7.05	4.29 7.71	4.40 7.35	4.69 7.50	3.77 6.17	4.46 6.98	4.76 8.18	3.76 5.93	3.30 5.95	3.67 6.20	3.94 6.18	4.07 6.37
4	3.90 6.91	4.64 8.23	4.05 7.89	4.04 6.66	3.49 5.70	3.91 6.46	4.52 7.60	4.52 7.29	3.05 5.82	4.59 5.41	4.01 5.99	3.34 5.60
5	3.63 5.93	4.27 7.73	4.92 8.58	4.64 7.86	3.76 6.05	4.10 6.77	4.40 7.91	4.17 7.33	3.67 7.33	5.07 8.05	3.83 6.36	3.63 6.43
6	3.16 5.59	3.55 6.45	4.08 7.04	4.73 7.98	3.61 5.89	3.66 6.15	4.39 7.36	3.84 6.28	4.26 7.61	4.36 5.58	3.79 5.46	4.09 6.10
7	3.18 5.32	3.50 5.87	4.23 7.02	4.39 6.98	3.28 4.97	3.56 6.06	4.94 8.06	4.06 6.81	4.32 7.37	4.86 7.51	3.96 6.23	3.74 6.38
8	3.42 5.52	3.35 5.71	4.07 7.05	4.66 7.28	3.72 5.78	3.84 6.10	4.44 7.50	3.40 5.97	4.07 6.99	4.93 7.57	3.93 6.13	3.04 4.96
9	3.97 6.03	3.61 6.01	4.67 7.64	4.49 6.95	3.75 5.94	4.11 6.83	4.64 7.92	2.99 5.07	4.37 7.25	5.02 8.23	3.56 5.75	3.59 6.03
10	3.40 5.66	3.71 6.29	4.27 7.17	4.42 6.95	4.13 7.31	4.20 6.86	4.66 7.59	3.61 6.33	3.99 7.26	4.44 7.75	3.86 6.14	3.99 6.26
11	3.39 5.61	3.64 6.30	3.84 6.60	4.51 7.86	4.01 7.18	4.21 8.23	4.77 8.23	3.95 7.11	4.17 7.52	4.39 7.73	4.17 7.20	4.17 7.08
12	4.43 7.07	3.70 6.17	3.82 6.87	4.45 7.17	3.59 6.53	4.37 8.75	4.45 8.74	4.08 7.17	3.72 7.28	4.56 7.73	4.10 6.90	3.07 6.12
13	4.52 7.10	3.79 6.33	4.41 7.03	4.57 7.93	3.94 7.06	4.47 8.53	4.42 8.02	3.92 6.70	3.86 7.20	4.77 7.67	4.99 7.82	3.91 7.34
14	4.46 7.16	4.09 7.22	4.39 7.73	4.31 7.31	4.29 7.08	4.47 8.15	4.37 7.69	3.44 6.62	3.82 7.05	4.63 7.87	4.36 7.39	3.79 6.33
15	3.46 8.85	4.42 5.20	4.29 7.52	4.08 6.67	3.96 6.54	3.96 7.10	3.89 6.86	4.11 7.58	3.73 6.89	4.03 7.16	4.09 7.03	3.82 7.30
16	5.13 8.37	3.89 7.05	4.26 6.99	4.32 6.93	3.78 6.72	3.54 6.46	4.27 7.79	4.12 7.32	4.13 7.24	4.47 7.84	3.41 5.96	3.55 6.70
17	4.23 6.89	3.87 6.82	4.23 7.14	4.58 7.73	3.19 6.06	3.49 6.63	3.91 7.34	4.41 7.53	4.21 7.41	4.61 7.51	4.27 7.17	4.06 7.00
18	4.38 6.72	4.12 7.38	4.39 7.55	3.92 6.70	4.84 8.85	3.94 6.75	4.38 7.43	4.24 7.32	3.96 7.04	4.29 6.96	4.52 7.73	4.19 7.30
19	3.85 6.59	4.28 7.03	4.69 8.06	5.16 8.78	4.46 7.54	4.41 8.15	4.68 7.51	4.37 7.19	4.15 7.47	4.91 7.96	4.68 8.07	5.13 8.31
20	3.61 6.20	4.22 7.65	4.42 8.45	5.09 8.72	3.66 7.09	4.22 7.72	4.06 7.06	3.97 7.53	3.89 7.36	4.46 6.91	4.44 6.87	4.52 8.17

MAP B. Wheat Yields, Upper Figures Grains, Lower Figures Straw,

13	14	15	16	17	18	19	20	21	22	23	24	25
4.58 7.23	3.92 6.33	3.64 5.11	3.66 5.96	3.57 5.12	3.51 5.05	4.27 6.54	3.72 5.47	3.36 4.76	3.17 4.95	2.97 4.53	4.23 6.08	4.53 6.78
4.05 6.57	3.97 6.03	3.61 5.58	3.82 5.80	3.44 5.00	3.92 5.83	4.26 8.61	4.36 6.14	3.69 5.56	3.53 5.09	3.14 5.11	4.09 5.91	3.94 5.68
3.73 6.02	4.58 7.23	3.64 5.86	4.07 6.74	3.44 5.56	3.53 4.91	4.20 6.55	4.31 6.44	4.33 6.17	3.66 6.15	3.59 5.41	3.97 6.28	4.38 7.49
4.06 6.19	3.19 6.56	3.75 4.62	4.54 7.08	3.97 6.03	3.77 5.79	4.30 5.95	4.10 5.96	3.81 6.13	3.89 5.92	3.32 4.62	3.46 5.41	3.64 6.55
3.74 6.13	4.14 5.98	3.70 7.67	3.92 6.14	3.79 5.33	4.29 5.58	4.22 6.15	3.74 5.76	3.55 5.89	3.67 5.45	3.57 5.24	3.96 5.60	4.31 6.56
3.72 6.03	3.76 5.49	3.37 5.00	4.01 5.99	3.87 5.57	4.35 6.09	4.24 5.88	3.58 5.61	4.20 5.92	3.94 5.87	4.24 5.82	3.75 5.50	4.29 6.15
4.33 6.79	3.77 5.48	3.71 5.66	4.59 7.28	3.97 6.03	4.38 6.24	3.81 5.69	4.06 6.25	3.42 5.45	3.05 4.57	3.44 4.56	2.78 4.28	3.44 5.68
3.72 5.97	3.93 6.07	3.71 5.79	4.76 6.49	3.83 6.29	3.71 5.91	3.54 5.21	3.66 5.78	3.95 5.92	3.84 5.66	3.76 5.24	3.47 5.59	4.24 7.26
4.05 6.82	3.96 6.35	3.75 5.12	4.73 8.64	4.24 6.45	4.21 6.29	3.85 6.15	4.41 6.15	4.21 6.04	3.63 5.81	4.17 5.58	3.44 4.81	4.55 6.32
3.37 6.25	3.47 5.78	3.09 5.47	4.20 6.49	4.09 6.16	4.07 6.18	4.09 5.47	3.95 6.11	4.08 7.00	4.03 5.72	3.97 5.65	2.84 4.10	3.91 5.96
4.09 7.28	3.29 5.71	3.37 6.44	3.74 8.63	3.41 5.78	3.86 6.14	4.36 7.39	4.54 7.46	4.24 7.20	4.08 6.54	3.89 5.98	3.47 5.84	3.29 5.65
3.99 7.13	3.14 5.05	4.86 6.39	4.36 7.26	3.51 6.11	3.47 5.90	3.94 6.68	4.47 7.84	4.11 6.95	3.97 6.47	4.07 5.80	3.56 6.38	3.83 6.29
4.09 7.72	3.05 5.70	3.39 5.86	3.60 6.27	4.13 6.87	3.89 6.23	3.67 6.20	4.54 7.33	4.11 6.64	4.58 6.79	4.02 6.35	3.93 5.69	4.33 7.11
3.56 6.69	3.29 5.71	3.64 6.36	3.60 5.84	3.19 5.87	3.80 6.14	3.72 6.34	3.91 6.96	3.35 6.27	4.11 6.64	4.39 6.11	3.47 5.78	3.93 6.07
3.57 6.55	3.43 5.38	3.73 8.58	3.39 6.42	3.08 5.42	3.48 5.52	3.05 5.20	3.65 6.60	3.71 6.29	3.25 6.37	3.69 5.18	3.43 5.82	3.38 5.68
3.16 5.84	3.47 5.84	3.30 5.70	3.39 5.80	2.92 4.95	3.23 5.33	3.25 5.25	3.86 6.64	3.22 5.40	3.69 5.93	3.80 5.70	3.79 6.21	3.63 5.99
3.75 6.31	3.91 6.21	3.57 5.99	3.45 6.05	3.05 7.64	3.68 5.82	3.52 5.85	3.91 6.71	3.87 6.13	3.87 7.50	4.21 5.48	3.68 6.01	4.06 6.88
4.49 7.57	3.82 6.37	3.60 6.34	3.14 5.48	2.73 4.77	3.09 5.41	3.66 5.84	3.77 6.98	3.48 6.14	3.76 6.11	3.69 5.43	3.84 6.35	3.67 6.33
4.19 6.93	4.41 6.78	3.54 5.58	3.01 5.68	2.85 4.96	3.36 6.14	3.85 6.15	4.15 6.85	3.93 6.57	3.91 6.09	4.33 6.04	4.21 6.98	4.19 6.93
3.70 6.80	4.28 6.97	3.24 5.95	3.29 5.58	3.48 5.52	3.49 5.82	3.68 6.76	3.36 6.08	3.71 6.35	3.54 6.21	3.59 4.66	3.76 6.36	3.36 6.33

on Rothamsted Acre.

whence

$$r_{p_1p_2} = \frac{42.721685 - (6.515)^2}{.805341} = .343 \pm .027.$$

Illustration 3. *Influence of Substratum Heterogeneity upon Yield of Grain and Nitrogen Content in Experimental Plots of Wheat.*

Table III is condensed from Map C of Montgomery[15]

532 2.13	514 2.06	525 2.20	501 196	534 2.10	486 2.03	483 2.19	451 2.06	395 2.08	440 2.13	432 2.03	441 2.11	410 2.15	470 2.14
612 2.16	510 2.05	460 206	530 2.00	538 2.09	471 1.98	439 2.17	446 2.14	424 2.13	430 1.96	424 1.97	405 2.01	436 1.98	472 2.04
515 2.10	480 2.20	485 2.08	534 2.06	423 2.05	421 2.11	446 2.15	393 2.02	414 2.09	421 1.97	422 1.95	423 2.03	380 1.96	432 2.02
553 2.03	542 2.00	583 2.11	524 2.13	420 2.08	436 2.05	428 2.18	400 2.17	383 2.08	434 1.98	395 1.94	421 2.06	490 1.95	467 2.00
575 2.12	532 2.06	550 2.09	548 2.05	480 2.04	432 2.00	420 2.04	452 2.01	460 1.97	449 1.92	474 2.11	443 2.02	484 1.94	494 2.10
576 2.14	559 2.13	353 2.07	530 2.21	576 2.05	583 2.07	566 2.15	517 2.04	530 2.13	346 1.98	495 1.97	474 1.88	434 1.86	573 2.05
548 2.06	513 2.09	533 2.05	517 2.14	503 2.04	580 2.04	514 2.03	632 2.25	740 1.86	641 1.97	506 2.04	495 2.02	560 1.99	575 1.95
550 2.01	463 2.18	550 2.08	540 2.11	497 2.00	424 2.18	519 1.95	606 2.14	756 2.44	656 2.00	584 2.10	623 1.93	599 2.00	695 1.94
465 2.09	456 2.17	487 2.01	343 2.26	628 2.24	728 2.13	616 2.05	620 2.16	724 2.13	675 1.95	647 2.09	710 1.96	711 1.98	633 2.01
545 2.00	577 1.97	583 1.99	515 2.06	515 2.27	535 2.23	467 2.21	577 2.26	581 2.15	648 2.21	707 2.22	738 2.11	717 2.11	621 2.28
504 2.02	657 2.31	760 2.14	551 1.93	528 2.07	558 2.18	575 2.11	531 2.05	686 2.10	656 1.97	716 2.19	739 2.02	730 2.08	658 2.03
582 2.00	596 2.01	595 2.00	622 2.01	644 2.06	541 2.08	584 1.96	673 1.92	676 2.04	712 2.32	666 2.11	688 2.04	639 2.04	555 2.06
668 2.08	662 1.98	613 2.07	693 2.10	666 2.20	643 2.03	570 2.02	674 2.16	661 2.00	742 2.12	802 2.21	634 2.22	634 2.06	634 2.07
661 2.07	582 2.03	590 2.03	560 2.07	585 2.14	576 2.09	500 2.08	542 2.17	557 2.05	538 2.15	500 2.29	588 2.16	685 2.16	587 2.16
730 2.18	650 2.05	650 2.08	586 2.22	533 1.97	617 2.04	561 2.15	496 227	527 2.23	637 2.18	385 2.11	585 2.09	625 1.99	637 2.06
575 1.99	495 2.04	502 2.08	584 2.16	716 2.26	725 2.10	563 2.11	477 2.14	513 2.14	649 2.03	547 2.06	488 2.06	512 1.93	426 2.00

MAP C. Yields of Grain in Grams and Percentage of Nitrogen in Montgomery's Wheat Plots. The Upper Entries are Yield in Grams of Grain, the Lower are Percentage Nitrogen Content.

[15] Montgomery, E. G., "Variation in Yields and Method of Arranging Plots to Secure Comparative Results," *Ann. Rep. Neb. Agr. Exp. Sta.*, 25, 164–177, 1912.

by combining adjoining plots 2×2. The following are the numerical values.

For grains produced,

$$S(p) = 123429, \quad S(p^2) = 70112319, \quad N = 224,$$
$$\bar{p} = 551.022, \quad \sigma_p{}^2 = 9375.826,$$
$$S(C_p{}^2) = 277945243, \quad m[n(n-1)] = 642,$$
$$[S(C_p{}^2) - S(p^2)]/m[n(n-1)] = 309275.184,$$

whence

$$r_{p_1p_2} = .603 \pm .029.$$

For percentage nitrogen,

$$S(p) = 465.29, \quad S(p^2) = 968.3721, \quad N = 224,$$
$$\bar{p} = 2.077187, \quad \sigma_p{}^2 = .008383,$$
$$S(C_p{}^2) = 3868.5047, \quad m[n(n-1)] = 672,$$
$$[S(C_p{}^2) - S(p^2)]/m[n(n-1)] = 4.315673,$$

and

$$r_{p_1p_2} = .115 \pm .044.$$

TABLE III

COMBINATION PLOTS OF MONTGOMERY'S WHEAT, 2×2-FOLD GROUPING AS INDICATED BY HEAVY LINES IN MAP C

2,168 8.40	2,016 8.22	2,029 8.20	1,819 8.56	1,689 8.30	1,702 8.12	1,788 8.31
2,090 8.33	2,126 8.38	1,700 8.29	1,667 8.52	1,652 8.12	1,661 7.98	1,769 7.93
2,242 8.45	1,981 8.42	2,071 8.16	1,955 8.24	1,785 8.00	1,886 7.98	1,985 7.95
2,074 8.34	2,140 8.38	2,004 8.26	2,271 8.37	2,793 8.27	2,208 8.09	2,429 7.88
2,043 8.23	1,928 8.32	2,406 8.87	2,280 8.68	2,628 8.44	2,802 8.38	2,682 8.38
2,339 8.34	2,528 8.08	2,271 8.39	2,363 8.04	2,730 8.43	2,809 8.36	2,582 8.21
2,573 8.16	2,456 8.27	2,470 8.46	2,286 8.43	2,498 8.32	2,524 8.88	2,540 8.45
2,450 8.26	2,322 8.54	2,591 8.37	2,097 8.67	2,326 8.58	2,005 8.32	2,200 7.98

Illustration 4. *Influence of Substratum Heterogeneity upon the Yield of Experimental Plots of Timothy Hay.*

I take as a final illustration of the application of the criterion of substratum heterogeneity here proposed, the plot data for timothy hay published by Holtermarck and Larsen, *loc. cit.* By combining their plots into groups of 4 Table IV is secured,

$$S(p) = 4268.8, \quad S(p^2) = 77968.50, \quad N = 240,$$
$$\bar{p} = 17.787, \quad \sigma_p^2 = 8.503,$$
$$S(C_p^2) = 309491.48, \quad m[n(n-1)] = 720,$$

whence

$$r_{p_1p_2} = .609 \pm .027.$$

TABLE IV

COMBINATION PLOTS 2 × 2, SHOWING YIELDS OF TIMOTHY HAY SECURED IN THE EXPERIMENT OF LARSON

The original field is not mapped here

87.4	99.0	78.5	65.8	67.2	63.3
76.4	70.2	75.0	73.1	67.7	59.7
76.9	65.2	64.2	89.7	72.1	64.3
65.1	54.1	66.4	98.9	83.3	64.3
57.9	64.7	61.1	88.6	72.2	64.8
73.0	55.6	62.2	75.6	82.8	71.1
58.1	72.1	67.2	60.2	77.5	75.6
71.7	67.0	54.3	64.8	81.6	75.2
68.8	70.4	61.7	81.2	72.8	61.4
77.5	79.6	66.9	83.4	73.9	68.5

B. *Cases in which the Combination Plots Vary in Size*

In the foregoing illustration the combination plots have been of uniform size, *i. e.*, have contained each the same number of ultimate plots. It may be desirable or necessary to have some of the combination plots smaller than the others. Thus the wheat field of Mercer and Hall is

laid out in a 20×25 manner. This permits only 2×5, 4×5 or 5×5 combinations of the same size throughout. Montgomery's experiment comprises an area of 16×14 plots which may be combined in only 2×2 or 4×2 equal areas suitable for calculation. In each of these cases other groupings are desirable.

The formulæ are quite applicable to such cases: the arithmetical routine is merely a little longer. The formula is again

$$r_{p_1p_2} = \frac{\{[S(C_p^2) - S(p^2)]/S[n(n-1)]\} - \bar{p}^2}{\sigma_p^2},$$

but $\bar{p}$ and σ_p are obtained by a $(n-1)$-fold weighting of the plots,[16] where n is the number of ultimate plots in the combination plot to which any p may be assigned, *i. e.*,

$$\bar{p} = S[(n-1)p]/S[n(n-1)],$$

$$\sigma_p^2 = \frac{S[(n-1)p^2]}{S[n(n-1)]} - \left(\frac{S[(n-1)p]}{S[n(n-1)]}\right)^2.$$

The point may be illustrated in detail on the wheat data of Mercer and Hall. I adopt a combination by twos from north to south, *i. e.*, arrange the data in 10 rows of combination plots instead of 20 rows of ultimate plots. From east to west there are 25 rows of ultimate plots; these can be only reduced to a 2×2-fold grouping for the first 22 rows. The lines of division are indicated on Map B by marginal arrows.

Row 23–25 must be thrown into combination plots each of 6 units. The possible permutations within a combination plot are $1/2\, n(n-1)$, but since the surfaces are rendered symmetrical, the total permutations for the whole field is $S[n(n-1)]$. There are only two sizes of combination plots, of which 110 have 4 and 10 have 6 ultimate plots each. Thus the weighted population N is

16 That is, each ultimate plot is multiplied by the number less one of the plots in the combination plot to which it is assigned.

$$S[n(n-1)] = (110 \times 4 \times 3) + (10 \times 6 \times 5) = 1620.$$

In the calculation of the weighted means and standard deviations each entry, and the square of each entry, in the first 22 rows must be weighted in an $(n-1)$-fold $=$ 3-fold manner, while those for the last three rows must be weighted in a 5-fold manner.[17]

The numerical values are:

For grain,

$$S[(n-1)p] = 6378.72, \quad S[(n-1)p^2] = 25452.4154,$$
$$\bar{p} = 3.937, \quad \sigma_p^2 = .207610,$$
$$S(C_p^2) = 33129.7080, \quad S(p^2) = 7900.6790,$$

whence

$$r_{p_1p_2} = .354 \pm .026.$$

Note that $S(p^2)$ is constant for all groupings.

For straw,

$$S[(n-1)p] = 10474.52, \quad S[(n-1)p^2] = 69042.7194,$$
$$\bar{p} = 6.466, \quad \sigma_p^2 = .813000,$$
$$S(C_p^2) = 89985.8976, \quad S(p^2) = 21623.9802,$$

whence

$$r_{p_1p_2} = .479 \pm .023.$$

Weighting has not materially changed the physical constants from the values given under illustration 2 above. The reasons for the conspicuous differences in the correlations will be taken up presently.

Montgomery's wheat data have been grouped into 2×2-fold combination plots in the illustration above. If we again combine the entries of Table III by twos, beginning at the upper left-hand corner, we have 12 combination plots each 4×4, or of 16 ultimate plots, and 4 combina-

17 Since each individual ultimate plot is compared once as a first (or as a second) number of a pair with every plot classed with it, the weighting of the individual plots for means and standard deviations is an $(n-1)$-fold one.

tion plots each of $2 \times 4 = 8$ ultimate plots. The method of dividing up the field is indicated by the marginal arrows on Map C.

$$S[n(n-1)] = (12 \times 16 \times 15) + (4 \times 8 \times 7) = 3104.$$

For grain,

$$S[(n-1)p] = 1707635, \quad S[(n-1)p^2] = 9683408.57$$

$$\bar{p} = 550.140, \quad \sigma_p{}^2 = 9311.307,$$

$$S(C_p{}^2) = 1023184887, \quad S(p^2) = 70112319,$$

whence

$$r_{p_1p_2} = .472 \pm .035.$$

For nitrogen,

$$S[(n-1)p] = 6458.63, \quad S[(n-1)p^2] = 13464.6031,$$

$$\bar{p} = 2.080744, \quad \sigma_p{}^2 = .008327,$$

$$S(C_p{}^2) = 14409.6095, \quad S(p^2) = 968.3721,$$

and

$$r_{p_1p_2} = .096 \pm .045.$$

Again the weighted means and standard deviations do not differ widely from those used above. The differences in the correlations will be discussed below.

In concluding this section it may be pointed out that all of the foregoing values are surprisingly high. They indicate clearly that the irregularities of an apparently uniform field may influence profoundly the yield of a series of experimental plots. They also bring out another interesting point. In the three cases in which two different characters were measured on the same species they show very different susceptibilities to environmental influence. Thus, for example, the correlation of mangold roots is $r = .346 \pm .042$ as compared with $r = .466 \pm .037$ for leaves. For grain on the Rothamsted field with a 4×5-fold grouping the correlation is $r = .186 \pm .029$ as compared with $r = .343 \pm .027$ for straw. For Montgomery's data for yield and composition the differences are

even more conspicuous. The correlation for per cent. nitrogen is $r = .115 \pm .044$ as compared with $r = .603 \pm .029$ for weight of grain produced.

This point will not be discussed in greater detail here, since the problem of the relative susceptibility of various characteristics of the individual to environmental influence has been the subject of experimental and statistical studies which have been under way for several years and will probably eventually be published.

III. ON THE NATURE OF THE REGRESSION OF ASSOCIATED PLOTS

The correlation coefficient is strictly valid as a measure of interdependence only when regression is linear, *i. e.*, when the means of the second variable associated with successive grades of the first lie in a sensibly straight line. The equation for the regression straight line

$$p_2 = \left(\bar{p}_2 - r_{p_1p_2}\frac{\sigma_{p_2}}{\sigma_{p_1}}\bar{p}_1\right) + r_{p_1p_2}\frac{\sigma_{p_2}}{\sigma_{p_1}}p_1$$

for the second on the first ultimate plot of the same combination plot reduces to

$$p = (\bar{p} - r\bar{p}) + rp,$$

when symmetrical tables in which $p_1 = p_2$, $\sigma_{p_1} = \sigma_{p_2}$ are used.

The testing of the linearity of regression in any individual case is rendered somewhat difficult by the necessity

TABLE V

YIELD OF GRAIN IN ROTHAMSTED WHEAT EXPERIMENT

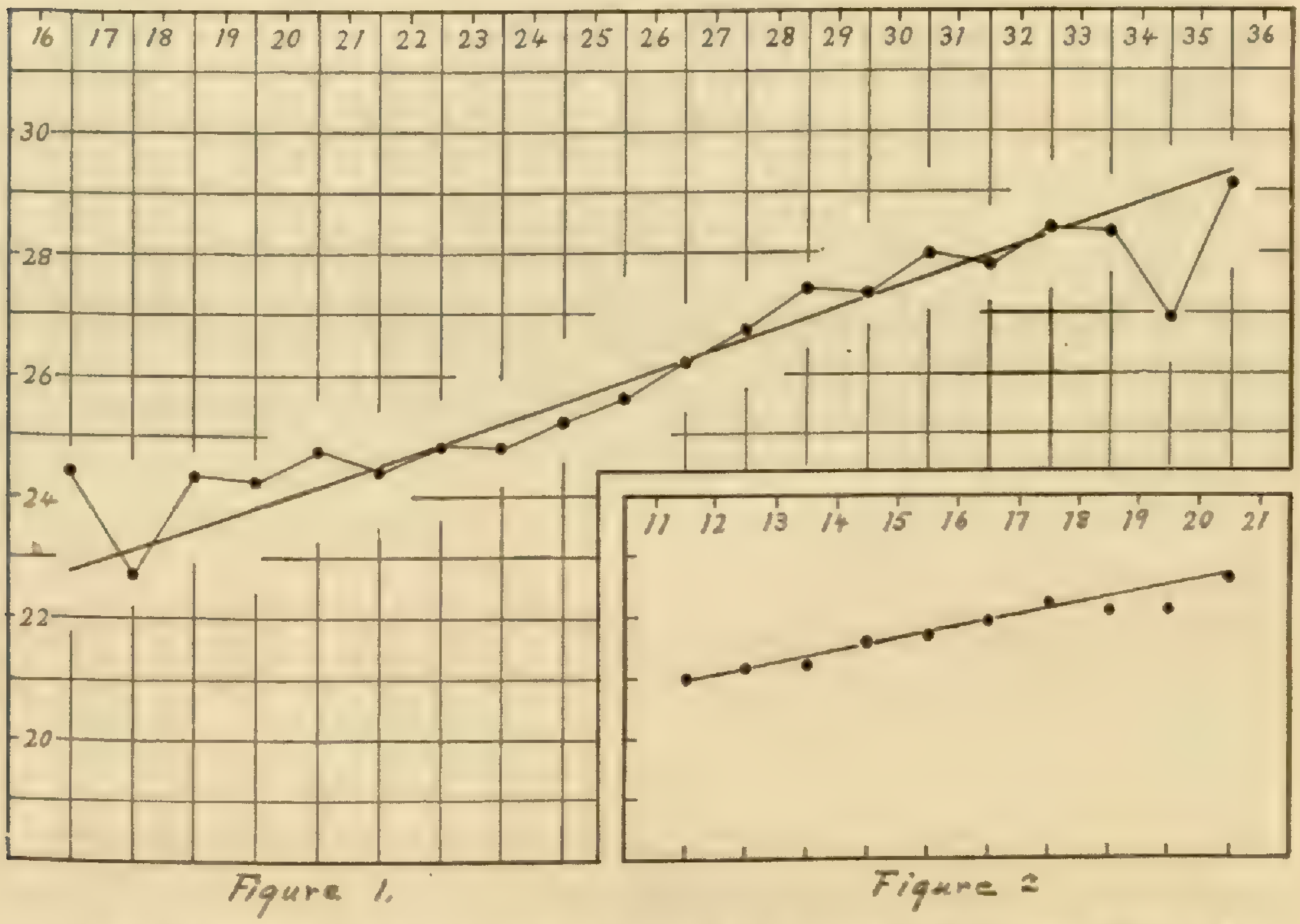
Yield of First Plot	Weighted Frequency	Mean Yield of Associated Plots	Yield of First Plot	Weighted Frequency	Mean Yield of Associated Plots
2.75–2.99	133	3.76	4.00–4.24	1786	3.99
3.00–3.24	475	3.78	4.25–4.49	1444	4.07
3.25–3.49	1026	3.81	4.50–4.74	703	4.04
3.50–3.74	1634	3.89	4.75–4.99	247	4.05
3.75–3.99	1919	3.93	5.00–5.24	133	4.16

of actually forming a correlation table from which to compute the means of arrays. The labor is greatly lessened by the use of some such scheme as that described for the formation of condensed correlation tables.[18]

TABLE VI

YIELD OF STRAW IN ROTHAMSTED WHEAT EXPERIMENT

Yield of First Plot	Weighted Frequency	Mean Yield of Associated Plots	Yield of First Plot	Weighted Frequency	Mean Yield of Associated Plots
4.00–4.24	19	6.11	6.50–6.74	608	6.56
4.25–4.49	19	5.68	6.75–6.99	817	6.69
4.50–4.74	133	6.08	7.00–7.24	779	6.86
4.75–4.99	171	6.07	7.25–7.49	665	6.84
5.00–5.24	304	6.19	7.50–7.74	627	7.04
5.25–5.49	418	6.13	7.75–7.99	323	6.96
5.50–5.74	722	6.18	8.00–8.24	247	7.14
5.75–5.99	1121	6.20	8.25–8.49	57	7.09
6.00–6.24	1273	6.31	8.50–8.74	152	6.75
6.25–6.49	969	6.38	8.75–8.99	76	7.28



FIGS. 1 AND 2. Mean Yields of Grain and Straw on Ultimate Plots Associated in the Same Combination Plots of a Given Yield. Rothamsted Wheat. Empirical Means and Fitted Straight Line. Units are Quarters of a Pound.

18 Harris, J. Arthur, "On the Formation of Condensed Correlation Tables when the Number of Combinations is Large," AMER. NAT., 46, 477–486, 1912.

For the 5×4 grouping of the 500 wheat plot of Mercer and Hall I find the values given in Tables V–VI.

For the regression of the second on the first plot the equations are:

For grain, g, $g_2 = 3.214 + .186\, g_{\bar{1}}$.
For straw, s, $s_2 = 4.280 + .343\, s_{\bar{1}}$.

Figs. 1 and 2 exhibit the usual irregularities of sampling in the means, but show no certain departure from linearity.

TABLE VII

YIELD OF GRAIN IN MONTGOMERY'S WHEAT EXPERIMENT

Yield of First Plot	Weighted Frequency	Mean Yield of Associated Plots	Yield of First Plot	Weighted Frequency	Mean Yield of Associated Plots
325–374	9	516.88	575–624	111	579.82
375–424	63	440.22	625–674	90	616.21
425–474	93	471.23	675–724	45	656.37
475–524	108	540.24	725–774	30	628.80
525–574	120	548.24	775–824	3	574.00

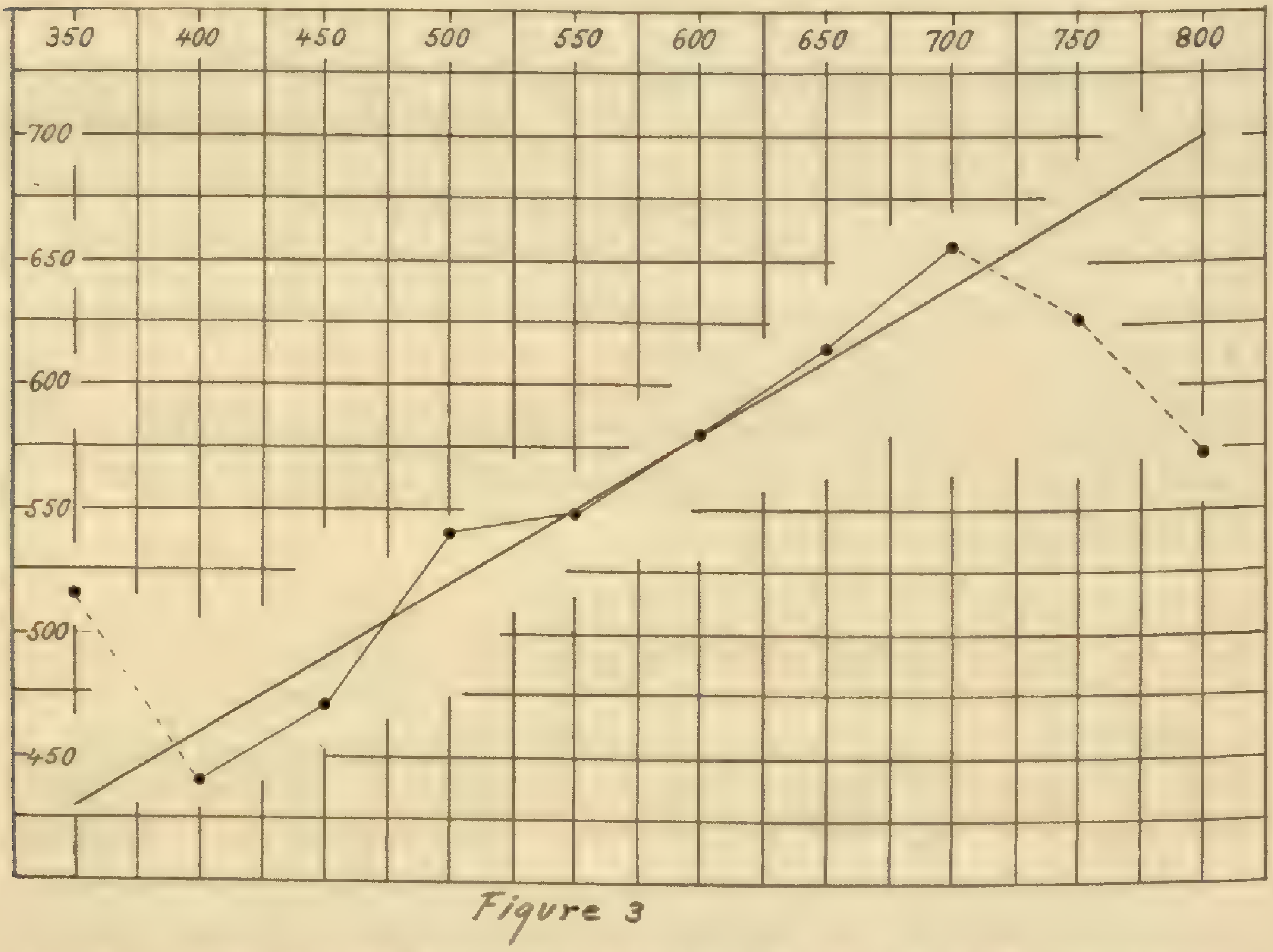


FIG. 3. Grain Yields in Nebraska Wheat. See Figs. 1–2 for Explanation.

Table VII gives the first plot character, weighted frequencies and empirical means for associated plots for 2×2-fold combinations from Montgomery's grain yield data in wheat.[19]

The equation is

For grain, g, $g_1 = 218.993 + .603\, g_1$.

The graph figures indicate sensible linearity.

IV. Influence of Number of Ultimate Plots Combined

If an experimental field exhibit irregularities of conditions which influence in a measurable degree the yield of

TABLE VIII

5×2–Fold Combination of Plots of Rothamsted Wheat

The divisions of the field are indicated by the double vertical lines and the arrows along the right margin in map B.

41.11 68.19	42.51 66.59	40.32 62.22	38.53 59.52	36.65 54.45
41.78 71.17	40.54 65.82	38.31 60.62	40.23 61.01	38.05 60.13
40.35 69.10	41.92 70.37	37.77 60.65	40.01 58.10	39.48 58.00
37.80 61.50	42.42 69.94	37.84 59.46	40.31 61.17	35.39 54.21
40.42 65.95	42.03 71.09	36.69 59.97	41.84 64.09	38.83 56.99
39.38 67.36	42.67 78.49	38.25 65.30	39.66 69.19	38.51 63.10
42.77 71.95	42.17 75.20	38.07 66.92	38.05 64.05	40.22 63.45
41.59 70.84	40.25 72.24	35.53 64.88	33.30 57.13	35.59 58.57
41.75 71.84	41.44 71.92	40.12 67.99	34.00 60.55	38.13 62.36
43.44 76.11	43.12 74.86	42.13 70.43	34.52 59.54	38.53 62.52

[19] Because of the many differences in the two experiments it is inadvisable to attempt drawing the regressions lines in a strictly comparable form.

neighboring small experimental plots, this heterogeneity should become apparently less when expressed on a scale of correlation between plots as the number of ultimate plots combined increases. The reason for this condition is quite simple. If the irregularities are very local in nature they will influence in the same direction the yield of only a very few neighboring plots. If too many ultimate plots be combined the correlation will tend to vanish because of the increased frequency of association of unlike conditions due to the fact that the combination plots have been made so large that they themselves have become heterogeneous.

That these conditions have been observed in actual experimentation is shown by the following constants based on different groupings of the data used above.

Consider first the Rothamsted wheat. For a 4×5 grouping of the plots the results were found to be

For grain, $r_{p_1p_2} = .186 \pm .029$,
For straw, $r'_{p_1p_2} = .343 \pm .027$.

If the plots be grouped by fives from east to west and by twos from north to south, Table VIII is obtained. The values $S(p^2)$, $\bar{p}$ and σ_p are the same as in the preceding case.

$$m[n(n-1)] = 50 \times 10 \times 9 = 4500.$$

For grain, $S(C_p{}^2) = 78265.2822$, $r_{p_1p_2} = .214 \pm .029$.

For straw, $S(C_p{}^2) = 213939.8774$, $r_{p_1p_2} = .365 \pm .026$.

If the combination plots be made even smaller by grouping in a 2×2-fold manner for all but the last three north and south rows, where a 2×3-fold combination must be adopted, the results are, as illustrated above,

For grain, $r_{p_1p_2} = .354 \pm .026$,
For straw, $r_{p_1p_2} = .479 \pm .023$.

For Montgomery's wheat data the results for a 4×4-fold grouping (in as far as the nature of the records will permit) have been shown to be

For grain, $r_{p_1p_2} = .472 \pm .035,$
For nitrogen, $r_{p_1p_2} = .096 \pm .045,$

as compared with the following values for a 2×2-fold grouping

For grain, $r_{p_1p_2} = .603 \pm .029,$
For nitrogen, $r_{p_1p_2} = .115 \pm .044.$

Finally consider the constants deduced from the hay yields published by Holtermark and Larsen.

For a 2×2-fold grouping, $r_{p_1p_2} = .609 \pm .027,$
For a 2×4-fold grouping, $r_{p_1p_2} = .471 \pm .034,$
For a 2×8-fold grouping, $r_{p_1p_2} = .278 \pm .040.$

Thus for every species of plant and every character considered the correlation between associated ultimate plots decreases as the number of plots grouped increases.[20]

TABLES IX AND X

2×4–FOLD AND 2×8–FOLD COMBINATION OF THE DATA FOR PLOT YIELD IN TIMOTHY HAY, TABLES DERIVED FROM TABLE IV

163.8	169.2	153.5	138.9	134.9	123.0
142.0	119.3	130.6	188.6	155.4	128.6
130.9	120.3	123.3	164.2	155.0	135.9
129.8	139.1	121.5	125.0	159.1	150.8
146.3	150.0	128.6	164.6	146.7	129.9

305.8	288.5	284.1	352.8	310.4	264.5
260.7	259.4	244.8	289.6	305.8	280.7
285.2	284.9	251.6			

[20] Of course, the same effect would be produced if comparisons were drawn between tests for substratum heterogeneity on fields comparable in every regard except for the size of the ultimate plots. Possibly, this explains in part, at least, the striking differences in the correlations for grain yield found from the records of Montgomery and of Mercer and Hall.

The Rothamsted plots were 1/500th acre in area or 87.12 square feet. Montgomery's plots were $5.5 \times 5.5 = 30.25$ square feet, or only about 1/3 of the area of the Rothamsted plots.

V. Recapitulation and Discussion

If the methodical production of new varieties of animals and plants to be made possible by the laws discovered in experimental breeding is to be of material practical value, more attention must be given to the development of a standardized scientific system of variety testing. From the practical standpoint, nothing is to be gained by the formation of varieties of plants differing in discernible features of any kind unless some of these varieties can by rigorous scientific tests be shown to be of superior economic value.

It is equally true that if tests of fertilizers or of different methods of irrigation carried out on an experimental scale are to have any real value as a guide to a commercial practise, the differences in the experimental results must certainly be significant in comparison with their probable errors.

The problem of plot tests has several different phases, all of which must ultimately receive careful investigation. The purpose of this paper has been to consider one of the problems only. To what extent do the irregularities of an apparently homogeneous field selected for comparative plot tests influence the yield of the plots?

The question has been far too generally neglected, although indispensable to trustworthy results. It is obviously idle to conclude from a given experiment that variety *A* yields higher than variety *B*, or that fertilizer *X* is more effective than fertilizer *Y*, unless the differences found are greater than those which might be expected from differences in the productive capacity of the plots of soils upon which they were grown.

The first problem has been to secure some suitable mathematical criterion of substratum homogeneity (or heterogeneity). Such a criterion should be expressed on a relative scale ranging from 0 to 1, in order that com-

The 2×2-fold grouping of Montgomery's plots gives a correlation of $.603 \pm .029$ as compared with $r = .354 \pm .026$ for as nearly a perfect 2×2-fold grouping as the Rothamsted records permit.

parisons from field to field, variety to variety or character to character, may be directly made. It should also, if possible, offer no difficulties of calculation.

The criterion proposed is the coefficient of correlation between neighboring plots of the field. An exceedingly simple formula for the calculation of such coefficients has been deduced.

The method of application of this coefficient is here illustrated by four distinct series of experimental data.

The remarkable thing about the results of these tests is that in every case the coefficient of correlation has the positive sign and that in some instances it is of even more than a medium value. In short, *in every one of these experimental series the irregularities of the substratum have been sufficient to influence, and often profoundly, the experimental results.*

It might be objected that by chance, or otherwise, the illustrations are not typical of what ordinarily occurs in plot cultures. But they have been purposely drawn from the writings of those who are recognized authorities in agricultural experimentation, and who have given their assurance of the suitability of the fields upon which the tests were made.

For example, Mercer and Hall state the purpose of their research to be, "to estimate the variations in the yield of various sized plots of ordinary field crops which had been subjected to no special treatment and appealed to the eye sensibly uniform." Their mangolds "looked a uniform and fairly heavy crop for the season and soil," while in their wheat field "a very uniform area was selected, one acre of which was harvested in separate plots, each one five hundredth of an acre in area." The data of Larsen were drawn from an experiment "auf einer dem Auge sehr gleichmässig erscheinenden, 3 Jahre alten Timotheegraswiese." Montgomery's data were secured from a plot of land only 77×88 feet in size, which had been sown continuously to Turkey Red wheat for three

years, "and was of about average uniformity and fertility."

Nothing could, it seems to me, emphasize more emphatically the need of a scientific criterion for substratum homogeneity than the facts that correlations between the yields of adjacent plots ranging from $r = .115$ to $r = .609$ can be deduced from the data of fields which have passed the trained eyes of agricultural experimenters as satisfactorily uniform.

December 12, 1914

SHORTER ARTICLES AND DISCUSSION

A NOTE ON THE GONADS OF GYNANDROMORPHS OF DROSOPHILA AMPELOPHILA

FIVE gynandromorphs of *Drosophila amelophila* were sectioned and their gonads studied in order to determine whether the gonads corresponded to the secondary sex characters expressed by the somatoplasm. The specimens were either lateral or fore and aft gynandromorphs.

I. This gynandromorph arose from a cross between a white eyed fly and a fly of the wild type. On one side of the body the eye was red, the wing long, the sex comb lacking, and the abdomen characteristically female. The other side had a white eye, short wing, sex comb and male abdomen. The external genitalia were abnormal.

The fly would not mate, not only because of the abnormality of the genitalia, but because its mating instincts were indifferent. It was courted by males but, in turn, it itself did not court females.

Since the fly was externally a bilateral gynandromorph one would expect to find that the gonads on one side were male and on the other side female. This, however, was not the case. The gonads on both sides were male and the testes were filled with ripe spermatozoa.

II. This fly arose from a cross of cherry club vermilion with the wild type. The left side had a cherry eye, sex comb, long wing and an abdomen of the female type. The sex comb is characteristic of the male and the long wing of the female. The right side had a red eye, no sex comb, short wing, and abdomen of the male type. The absence of the sex comb is characteristic of the female while the short wing and dark abdomen on this side were male. This is lateral and, at the same time, a fore and aft gynandromorph. The left side was male anteriorly and female posteriorly while the right side was female anteriorly and male posteriorly. The gonads were male but immature. No ripe spermatozoa were seen.

III. The origin of this fly was the same as the last. Both

eyes were red, sex combs lacking, left wing long, and the abdomen characteristically male. The external genitalia were apparently half male and half female. This is not a fore and aft gynandromorph but a lateral one in which the parts involved are restricted to the abdomen and the posterior part of the thorax.

The fly was courted assiduously by males but it would not mate. The gonads on both sides were female and ripe eggs were present. It is probably true that the eggs could not be deposited on account of some defect in the oviducts.

IV. The origin of this fly was the same as the last two, *i. e.*, it came from a cross of cherry club vermilion with the wild type. The eyes were red, sex combs lacking; the wings were of the same length; the abdomen was divided into a female and a male side and the external genitalia were apparently half female and half male. Anteriorly the fly was female, and posteriorly it was half male and half female.

A male courted this gynandromorph as long as the male remained in front of it. When the male with one wing vibrating made a half circle to the tip of the abdomen, it immediately dropped its wing and turned and ran. Sections showed mature spermatozoa in both testes.

V. This gynandromorph arose from the cross of an abnormal form, a possible mutant, with the wild type. The eyes were red, but on one side there was a sex comb and a short wing, while on the other side the sex comb was lacking and the wing was long. The abdomen was characteristically female. The gonads were of the female type on both sides.

The conclusion, if one is justified in drawing a conclusion from so few data, is that the gonads of lateral gynandromorphs do not follow the separation of the somatic cells into a male and a female side, but are always the same on both sides, either male or female. Since the cells of an early embryo must be either male or female producing, we can understand why the gonads of a gynandromorph should be alike on both sides, regardless of the somatic condition, if we suppose that the gonads are derived from a single cell of the embryo.

F. N. Duncan

Columbia University

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THE CHROMOSOME VIEW OF HEREDITY AND ITS MEANING TO PLANT BREEDERS[1]

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Definite advice as to practical procedure must be based on a firm foundation of fact if the leaders in the applied science are to retain any confidence in those who lay the first stones in the pure science. At the same time, if it is clearly understood that science only approximates truth, that so-called "established laws" are only highly probable and never absolute, it can hardly be said to be unwise if an inventory of fact is taken at any time. The handwriting on the wall is never finished; some words are dim and the erasures and omissions are many, but that is no reason why one should not try to read it and to see what it directs if he has translated aright.

This preliminary justification of the title of this article is made because our present stock of facts regarding heredity points clearly to the chromosomes as vital parts of the mechanism, and I wish to emphasize some important practical deductions in case this position continues to become more firmly established.

A just and complete dissertation upon the rôle of the

[1] This paper is based upon two lectures delivered at Harvard University in 1914. I hope that any cytologists who may have their attention called to it will overlook the repetition of some well-known facts in the first few pages, as it is intended to be merely a general statement of a particular point of view with certain deductions that follow if it be accepted. I wish to thank Doctors O. E. White, T. H. Morgan and R. Goldschmidt for their kindness in giving me many suggestions, but in justice to them I should state that they are not responsible for the conclusions drawn.

chromosomes in heredity not only would fill many pages, but would expose numerous gaps in our present knowledge, gaps that leave several important questions in the balance. We shall assume frankly therefore that the chromosomes *are* the bearers of the determiners of practically all of the hereditary characters that have been investigated by pedigree culture methods, acknowledging freely our ignorance on many points, but maintaining that while no facts have been discovered which offer insurmountable arguments against the viewpoint taken, the following logical sequence of truths discovered at various times and by different methods of research make a pretty sound case upon which to base our practical conclusions.

Relative Importance of Nucleus and Cytoplasm

There are several reasons for believing that of the two parts of the cell, the nucleus and the cytoplasm, the former plays the greater rôle in heredity.

In general it is believed that the two parents contribute equally in the production of offspring—that the male and female contribution of potential characters is practically the same. If there were a difference it would be shown by divergent results in reciprocal crosses, but the investigations following Mendel's method make it probable that with the exception of sex and sex-linked characters, the results of reciprocal crosses are generally alike. This being true, it would appear that the principal basis of inheritance must be sought elsewhere than in the cytoplasm, for in most observed cases the sperm is very much smaller than the egg, and this difference is largely a difference in the amount of cytoplasm each carries. Is one not to look for some significance in this disparity in size? Strasburger, as well as other botanists, has even gone so far as to declare the male generative cell in certain angiosperms to be simply a naked nucleus that slips out of its cytoplasmic coat into the embryo sac, leaving the discarded coat behind, and that stimuli proceeding from the nucleus control the assimilation of food in the cell and determine even the character of the cytoplasm itself.

This belief may be too radical. The machine must have all of its parts to do proper work; and it may be, as Conklin suggests, that such characters as polarity, symmetry and localization of organ bases in the egg have their chief seat in the cytoplasm. This is only a possibility and not a fact, however, for one must admit that cytological investigation has not disclosed the presence of a material basis of heredity in the cytoplasm, though he may not be convinced that it is unimportant. Does the same statement hold for the nucleus?

The nuclear cavity contains four substances as they are ordinarily described in connection with morphological investigations. These are nuclear sap, linin, nucleolar material and chromatin.

Nuclear sap probably belongs as much to the cytoplasm as to the nucleus, and we know nothing as to its possible significance and importance within the nucleus.

Linin by some investigators is regarded as very similar to chromatin. Others (Strasburger) consider it to be the framework of the chromosomes, and the only real substance within the nuclear cavity that is continuous from generation to generation. It is a thread-like material staining lighter than chromatin upon which the chromosomes appear to be strung in the early prophases of nuclear division.

Nucleolar substance, though it stains in a different manner from chromatin, is considered by many to be chromatin-like in its nature. It is the substance of which the nucleoli are composed; but as these bodies become vacuolated and finally disappear during nuclear division, one is led to believe with Strasburger that they are temporary storehouses of some necessary food material.

Chromatin, however, as the material of which the chromosomes are composed, plays such a peculiar part in the activities of the cell, that hypotheses as to the meaning of its behavior are certainly more than shrewd guesses, as will be seen.

The chromosomes may be described as morphological

elements, of various shapes and sizes that are found within the nucleus; they are especially demonstrable as deeply staining bodies, definite in number for each cell at the period of division. In many cases in both plants and animals they have been found to be made up of small particles, the chromomeres, and various investigators have expressed the belief that these, too, are definite in number and play an important part in the larger collective entity, the chromosome.

Almost from their discovery, the chromosomes have had an especially important part assigned to them in the drama of heredity because of the previous philosophical deductions of Weismann. Weismann reasoned that if there were no reduction of heritable substance in the life cycle of an organism, it would pile up indefinitely because of the nuclear fusion at fertilization. He, therefore, predicted the discovery of some mechanism by which the character conserving substance would be divided. A few years later his prediction was verified in its important details by actual observation of the chromosome reduction in the formation of germ cells in *Ascaris*. From this discovery and from the facts that a specific number was found for the cells of each species, that all the cells of an individual appeared to possess the same number (except when they were halved at gametogenesis), that they were apparently permanent organs, that they were longitudinally halved in division so as to give each daughter cell the same number as well as an exact half of each chromosome possessed by the mother cell, investigators were early tempted to place upon chromosomes the whole burden of inheritance.

Our observations regarding chromosomes and the reduction divisions in plants now rest on a basis of cytological investigation of over 250 species, representing over 150 genera and divided among the four great groups of this kingdom. Montgomery's 1906 list of chromosome numbers in animals represents investigations on 185 species, comprised in about 170 genera, distributed among

nearly all the phyla of the animal kingdom. Sex chromosome studies have undoubtedly increased these figures for the animal kingdom to date, by hundreds of species.

Variation in chromosome number among the cells of an individual plant or animal is a recognized fact among cytologists, but this variation is not regarded as of particular significance, as commonly it is held to exist only among old cells, cells highly specialized, or, at any rate, cells which will never have anything in common with reproduction. To quote from Strasburger,

> the number of chromosomes in the nuclei of the somatic cells of both the sexual and the asexual generations have been found to vary. But so far as my experience goes, these observations are always to be observed in the nuclei of cells which are no longer embryonic, like those in an embryo or growing point, but which, on the contrary, are to some extent histologically specialized and are not destined eventually to give rise to reproductive cells. The determinate number is still more frequently departed from in nuclei which are definitely excluded from the sphere of reproduction.

In the reproductive cells, chromosome division is, on the other hand, very exact, and the numbers found, almost invariable, with one exception. This exception is the so-called accessory chromosome or chromosomes, that appear to be coupled with sex differentiation. And the very fact that such accessory chromosomes do exist and by their presence or absence parallel sex distribution, forms one of the most unanswerable arguments in favor of the chromosomes being the chief bearers of character determinants.

Morphological Individuality of the Chromosomes

The next topic to consider is whether there is sufficient evidence to support the idea that these bodies—the chromosomes—are morphological entities persisting from one cell generation to another.

Prochromosomes are deeply staining bodies found in the resting cell nuclei of plants, which probably correspond in number, but not in size, to the chromosomes which are found in the dividing nuclei. These bodies are

thought to represent the resting nuclear condition of the chromosomes. Prochromosomes have been found in at least sixty species of plants, and various structures comparable to them in many others. These investigations favor the thought that the chromosomes are persistent morphological entities; nevertheless they are not sufficient to establish the matter if there were no other data at hand.

There is a series of facts, however, which is more convincing. We are told that in addition to each species of animal or plant having in the larger part of its cells a specific number of chromosomes, there is a constant reappearance of the different shapes and sizes of these chromosomes in the same positions relative to one another during cell division after cell division.

Strasburger says: "The observation of such a series of stages of nuclear division as can be obtained by the laying open of embryo sacs in which development of endosperm tissue is commencing, makes it difficult to resist the impression that it is always the same chromosomes which make their appearance over and over again in the repeated divisions. In the prophase, the chromosomes are seen to appear in precisely the same position that they occupied in the preceding anaphase, and if the picture of the anaphase were proportionally enlarged, it would exactly correspond to that of the succeeding prophase."

The facts from which these general conclusions have been drawn can not be denied. Baltzer found odd-shaped chromosomes of similar shape in many maturing eggs of sea urchins. Boveri, Montgomery and later Schaffner pointed out a constant difference in the form and the size relations of the two chromosomes of *Ascaris megalocephala univalens*. Sutton thought he could recognize each individual chromosome in eleven consecutive cell generations of the maturing germ cells of the lubber grasshopper *Brachystola magna*. The so-called sex chromosome which has been found in so many insects and

other animals, is a clear case of constancy in appearance. In plants the same phenomenon has been observed. Rosenberg investigated the pollen mother cells of *Crepis virens* and in certain stages in division invariably found two long, two intermediate and two very short chromosomes. Division figures in the somatic cells showed the same differentiation, and in an examination of the nuclei of the pollen grain he found only one chromosome of each kind present. Such other species of this genus as have been investigated also show some variation in chromosome form, although it is not so striking as in *C. virens*. *Hieracium venosum*, exceptionally good material also investigated by Rosenberg, has shown the same thing. Edith Hyde remarks on the fact of the constant reappearance of certain chromosome forms among hundreds of division figures which she observed in *Hyacinthus orientalis*. Sauer mentions a very long chromosome constantly present in pollen mother cell preparations of the lily-of-the-valley, and Strasburger and Lutz found a large chromosome among many small ones in *Lychnis dioica*. In certain species of *Yucca* this chromosome differentiation takes on a dimorphic aspect, ten of the chromosomes being very large and about forty-five very small.

Taking into consideration all of these facts, of which hardly more than a random sample has been given, one is clearly justified in concluding that these cell characters are reproduced generation after generation. Why this constancy if they are not important?

Physiological Individuality of the Chromosomes

There is also considerable reason for believing that the various chromosomes of a cell may have different functions.

Boveri was the first to endeavor to test this hypothesis by allowing sea-urchin's eggs to be fertilized by two spermatozoa. Three nuclei, each with eighteen chromosomes, were thus present in the same egg, two male and one female. Although cytoplasmic division seemed to pro-

ceed normally, the chromosomes were usually distributed irregularly by a three-poled or a four-poled spindle. As a result three or four cells were produced at the first division of the doubly fertilized egg, instead of the two cells that arise after normal fertilization. Various abnormal larvæ were produced later. In such embryos, Boveri found the organism to be divided into definite regions, thirds or fourths, each part traceable to one of the three or four original cells, and the cells of each part differing from the cells of the other parts in their combination of chromosomes and usually in their chromosome number. In rare cases normal embryos were produced, but these were more commonly developed from a doubly fertilized egg which in its first division was three-celled, than from one in which it was four-celled. The thought occurs at once that three cells have a better chance than four cells in securing a full set of chromosomes, both as to number and kind. If the division were normal, each nucleus would receive a full set in the case of the chromosome distribution to three cells, but the division is usually irregular, and because of this irregularity each cell does not usually secure its normal set of chromosomes. Nevertheless it is clear that the embryo parts developed from the three-celled cleavage stand a much greater chance of being normal than those from the four-celled type, although through irregularities in division an eighteen-chromosome-celled region might be formed even where the first division was four-celled.

In some cases, the embryo was completely normal as regards skeleton and pigmentation in one or even two of its thirds, while the remainder was entirely lacking in these characters. Nearly normal embryos occurred which were perfect as to parts and specific characters, but individual variations which normally should have appeared in separate larvæ were present among the thirds. Asymmetrical larvæ also were formed.

More important still are the results Boveri obtained by isolating the three cells of the three-fold type and the

four cells of the four-fold type and allowing them to develop into larvæ. When the four cells of a four-celled stage of a normal embryo are separated, each cell produces a normal dwarf embryo alike in every respect, but the three- or four-celled embryos from double fertilized eggs, when treated in the same manner, never produce normal dwarfs even when the chromosome distribution has been numerically equal. Large numbers of larvæ brought into existence through this experiment showed all possible combinations of characters, just as all possible chromosome combinations were found in their nuclei, and from these and other data the conclusion is drawn that "not a certain number, but a certain combination of chromosomes is necessary to normal development, and this clearly points out that chromosomes have different qualities." In other words, the sea urchin has a set of eighteen chromosomes, each chromosome performing at least some different functions from its neighbors, making it necessary for the whole set to be present in order to insure normal development.

In further investigations, Boveri placed sea-urchin eggs which had been normally fertilized and were about to divide under pressure. As a result, division of the nucleus took place, but often no division of the cytoplasm. Such eggs on again dividing often formed more than two poles, resulting in inequalities in chromosome distribution and abnormal larval development. Boveri puts upon these cases an interpretation similar to that of the preceding experiments, as the irregular chromosome distribution seems to be all they have in common.

Morgan comments on Boveri's experiments as follows:

> The evidence makes probable the view that the different chromosomes may have somewhat different functions and that normal development depends on the normal interactions of the materials produced by the entire constellation of chromosomes.

Artificial parthenogenesis and experiments with enucleated eggs have proved that only one set of chromosomes is necessary to normal development of embryos, but it is

important, in considering these experiments, to note that two sets of similar chromosomes are present in a normal sexually produced organism.

Pairs of chromosomes of each shape and size (if they differ in shape and size) are *nearly* always found in the somatic cells—the exception being when the so-called accessory chromosomes are present. And since but one of each kind is found in the two gametes that fuse to form the new organism, it is only natural to suppose that one set was contributed by the maternal parent and the other by the paternal parent.

The numerous cases in which this phenomenon has been demonstrated are to many the most convincing evidence of some sort of a morphological individuality of the chromosomes. To them the fact implies pairs of freight boats loaded with the essential materials of life, to others—the minority—it is no more wonderful than the constant recurrence of other plant organs. At any rate, it has been shown that these sets of chromosomes continue an apparently independent existence for some time. Moenkhaus produced hybrids between the two species of fish, *Fundulus heteroclitus* with long straight chromosomes and *Menidia notata* with short curved chromosomes, and the early divisions of the fertilized egg showed clearly complete sets of chromosomes from each parent. Rosenberg obtained similar results in crosses between the two sundews, *Drosera longifolia,* which has forty small chromosomes, and *Drosera rotundifolia,* which has twenty large chromosomes. In some cases similar to the latter, where one parent contributes a greater number of chromosomes, it should be noted that the organism seems to have regulatory powers. The chromosomes unnecessary for a double set are either thrown out or take no part in the activities of cell division. For example, in the supposedly hybrid sundew, *Drosera obovata,* Rosenberg found that its thirty chromosomes behaved in the following peculiar manner. Ten of them paired with another ten, but the other ten remained unpaired and acted in a very abnormal fashion

in the reduction divisions. The ten pairs separated normally, one of each pair going to each pole; but the ten unpaired were irregularly distributed, sometimes nearly all of them going to one pole, sometimes most of them becoming lost in the cytoplasm and forming small nuclei. Embryos were produced in a very few cases and these only through back-crossing with pollen of *D. longifolia.* Unfortunately these embryos only developed through a few cell divisions.

These chromosome pairs have been distinguished by the name homologous chromosomes. For a long time it was thought that the paternal and the maternal set of chromosomes separated from each other bodily at the reduction division. Now it is believed to be only a matter of chance which chromosome of a pair passes to a particular daughter cell. There is some cytological evidence for this view, but the main argument in its favor is that this behavior is all that is necessary to fit nearly all the known facts of heredity, with the chromosomes playing the part of the active heredity machinery as will be seen shortly. This statement is true in a broad sense, but the word nearly is used because there is an exception to it. Chance apportionment of either member of a homologous pair of chromosomes to a daughter cell accounts for all facts of alternative (Mendelian) inheritance except where there are breaks in the correlation between characters usually inherited together. Since such breaks in correlation are common, it is clear that there must be a period when chromosome pairs have such an intimate relation that material can be exchanged. Many biologists believe that such a period is found during the maturation of the sex cells. The particular point at which such a conjugation or approximation of chromosome pairs takes place is called synapsis; it occurs as a part of the prophase or first stage of the reduction division. Some investigators have been unable to demonstrate any real chromosome fusion at this time, but all agree that there is an approximation between the two sets, and a chance for some kind of an exchange or interaction to take place.

Evidence of the physiological individuality of the chromosomes may be concluded by referring briefly to the so-called accessory chromosome. This fraction of a chromosome, whole chromosome, or in some cases, group of chromosomes, possesses no true synaptic mate, and therefore at reduction division two types of daughter cells are found. The presence or absence of the "accessory" is so closely associated with sex determination that most biologists now regard it as the morphological expression of a germinal sex determinant. The essential result of researches on this body may be summed up in the following words of Wilson.

> They have established the existence of a visible difference between the sexes in respect to these chromosomes, and have shown that it is traceable to a corresponding difference in the nuclei of the gametes of one sex or the other.

The simplest type of accessory chromosome, where the male possesses an unpaired chromosome which passes to one pole undivided in one of the spermatocyte divisions and hence enters but half the spermatozoa, was discovered by Henking (1891) in *Pyrrhocoris*. This work was confirmed in certain species of Orthoptera in 1902 by McClung, who advanced the hypothesis that the odd chromosome was a sex-determiner. Shortly afterward this was made more probable by Wilson and by Stevens who proved for several species of Hemiptera that the body cells of the males contain one less chromosome than the females. Two accessory or X chromosomes are present in the female, while but one is present in the male.

About the same time, both Wilson and Stevens independently discovered another kind of dimorphism in male germ cells of certain Hemiptera. Here the X chromosome of the male has a smaller synaptic mate Y. The body cells of the female, however, show two of the large X chromosomes. The sexes, therefore, both contain the same number of chromosomes, but have the same type of chromatin difference as was first discovered. The female is XX and the male XY.

Baltzer claimed in 1909 that in the sea urchins *Sphærechinus* and *Echinus* the sex with the dimorphic germ cells is the female instead of the male, but the work of Tennent has shown him to be in error and he has retracted the statement. There is, therefore, no undisputed cytological evidence demonstrating this type of dimorphic eggs; but since breeding results on certain species of birds and of lepidopters can be interpreted only on such an assumption, it is safe to assume that sooner or later they will be found.[2] Whether or not there are animals of this type, however, is of no particular importance in the present discussion. What we desire to emphasize is that a large number of animals, including man, have been shown to have a chromatic difference between the sexes, and that this difference is readily explained by the fact that the eggs are of a single type and the spermatozoa of two types.

In diœcious plants no such morphological differentiation has been found. But this fact does not negate the idea that the visible differences found in animals are really sex-determining differences. We have only to suppose that the dimorphism is primarily qualitative and secondarily quantitative. Indeed Wilson has found that the Y chromosome—the synaptic mate of the X—may vary in different species from a size equal to that of X until it disappears entirely, leaving X without a mate.

There is only one criticism in this whole matter. One may admit these cytological differences between the sexes, but hold that they are early appearances of secondary sexual characters. Morgan, von Baehr and Stevens have answered this impeachment. In the phylloxerans and aphids all the fertilized eggs produce females; males arise only by parthenogenesis, though females may arise in this manner. The cytological facts are as follows: Under favorable external conditions eggs develop without reduction and females are formed. Under unfavorable conditions one or two chromosomes (the sex determiners) are thrown out. If these eggs develop without fertilization

[2] Dimorphic eggs in Lepidoptera have recently been demonstrated by both Doncaster and Seiler.

males arise. The somatic condition of the females may therefore be termed XX and that of the males XY. If both reduced normally at any time, ordinary fertilization might be expected to give both males and females. But the spermatocytes without X degenerate, leaving only one type of functional spermatozoa, which produces females. Thus actual causal connection between the X chromosome and sex determination appears to have been demonstrated.

These are the main cytological arguments in favor of the chromosome view of heredity that seem to me to be insuperable. There are minor arguments both pro and con, which, as I said in the beginning, we have not space to consider. Instead it seems more profitable to show how Mendelian results interlock with those from cytology like the parts of a jig-saw puzzle.

CHROMOSOMES AND MENDELIAN INHERITANCE

The principal phenomena of Mendelian inheritance are: (1) characters that breed true; (2) uniformity of the population of the first hybrid generation in particular traits in which homozygous parents differed; (3) independent segregation of certain character determiners; (4) recombination of certain characters; (5) perfect coupling between certain characters; and (6) partial coupling between certain characters. Let us see how plausibly one can picture the mechanism through which such phenomena may result without imputing to the chromosomes any behavior that is not known to occur. To do this simply let the imagination portray a plant species having four chromosomes, each chromosome having three character determinants that can be followed through the breeding results that are obtained.

Our figures represent the immature germ cells of the plant just previous to the reduction division. Fig. 1 shows the germ mother cell with a duplicate set of hereditary determinants. The mature germ cells are exactly alike, therefore the plant breeds true to the characters concerned.

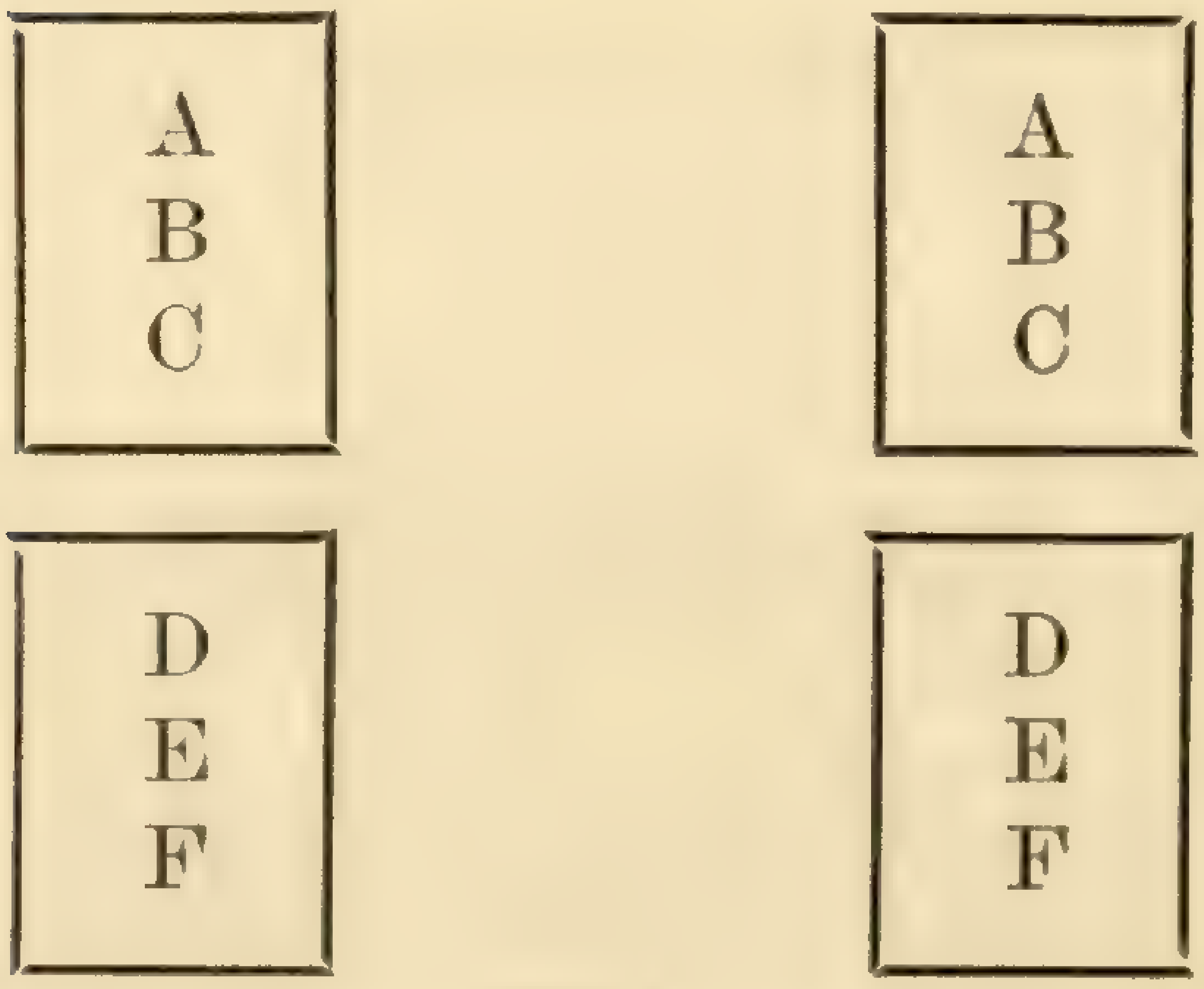

FIG. 1

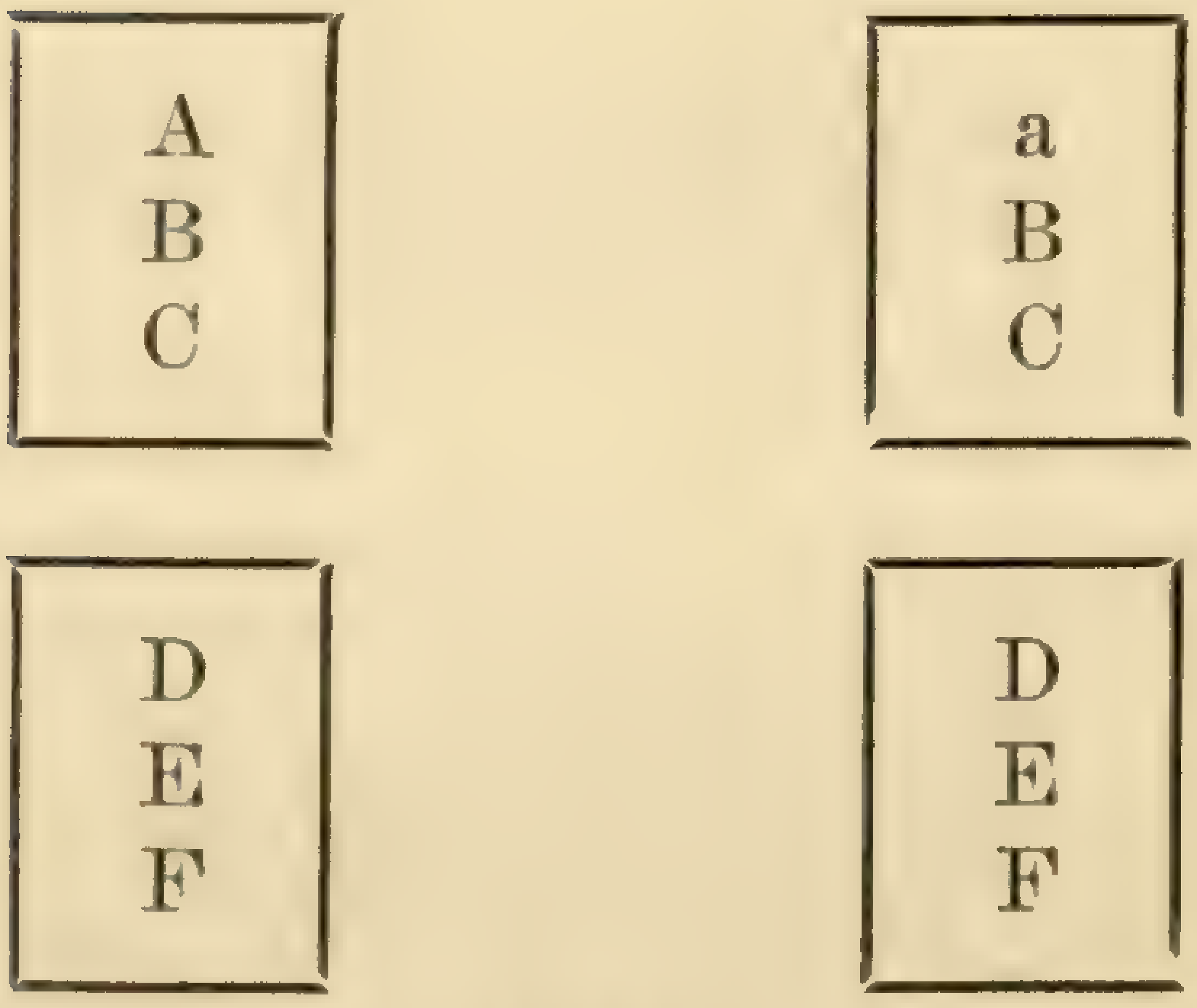

FIG. 2

Suppose, however, that a change in the germ plasm has occurred (Fig. 2) at some time or other. In one member of the first pair of chromosomes, determinant "A" has become "a." The mature germ cells differ from each other by one factor. For this reason the plant does not breed true, but gives a mono-hybrid Mendelian result.

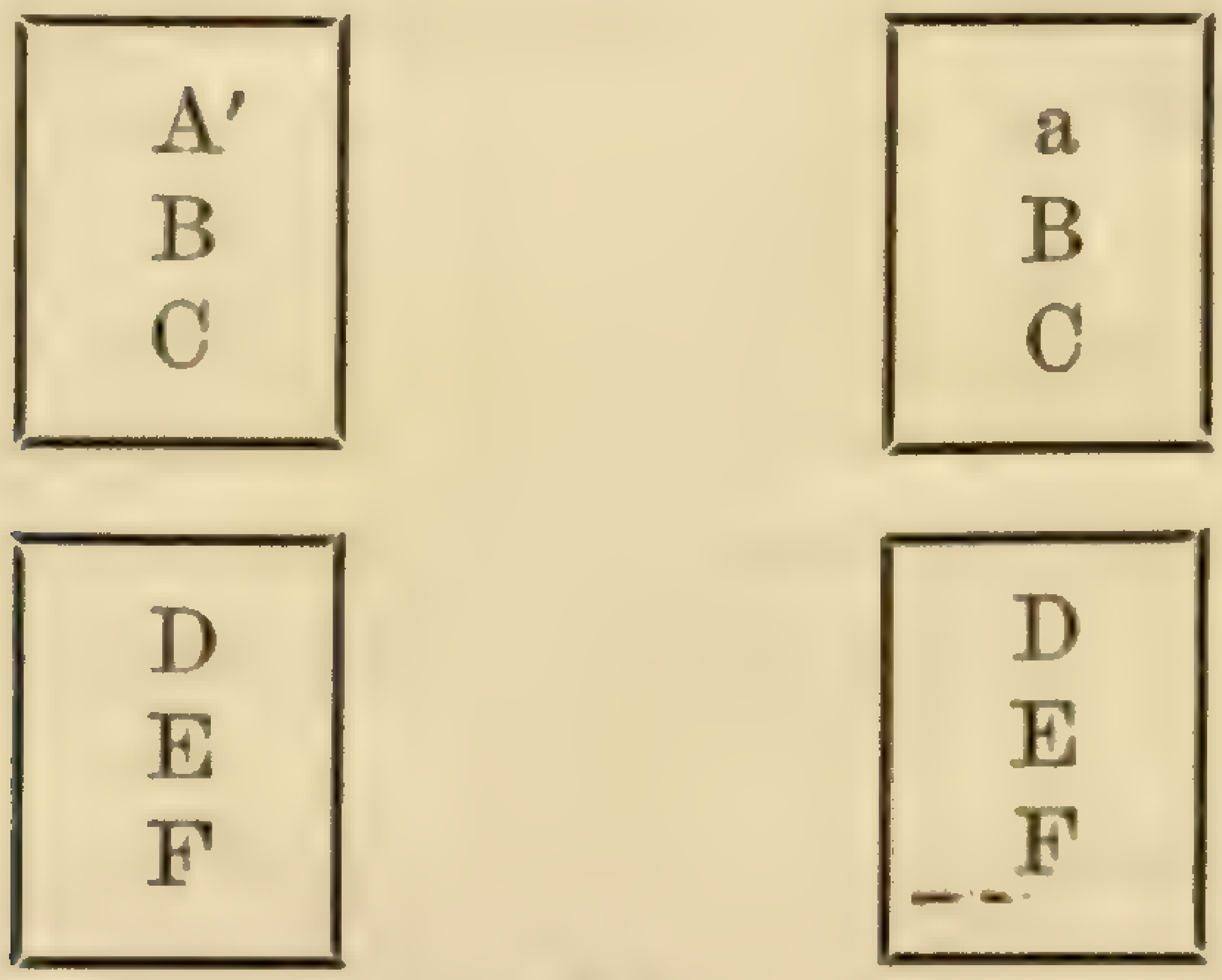

FIG. 3

Again, if such a change occurs that A becomes A′ (Fig. 3), a series of triple allelomorphs giving monohybrid results with each other, is formed. "A" is allelomorphic to "A′" or "a."

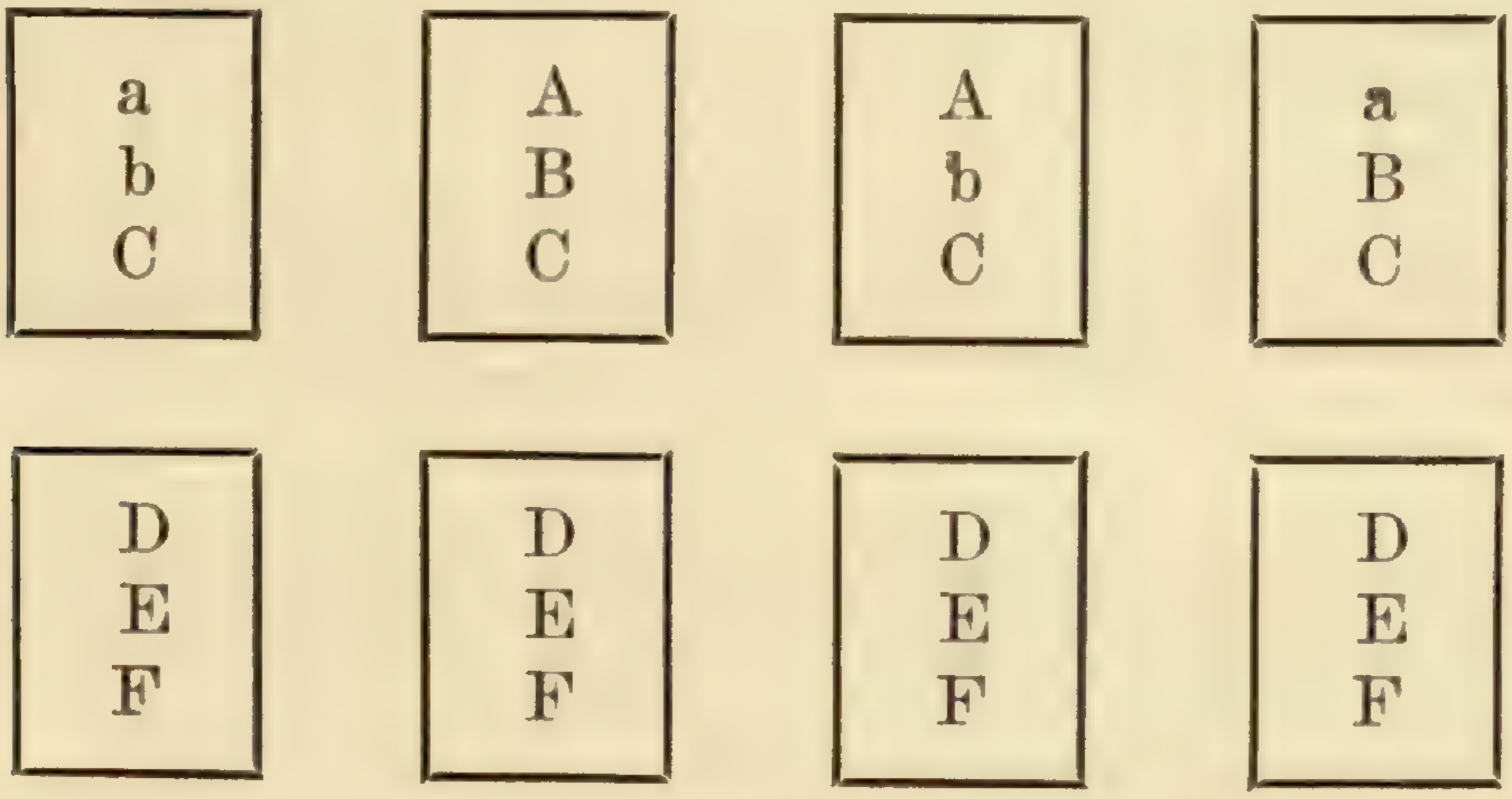

Fig. 4

But there are other character determinants in the first pair of chromosomes. What happens if both "A" and "B" become changed? There are two possibilities, as shown in the two parts of Fig. 4. If one of the members of the pair of homologous chromosomes becomes abC while the other remains ABC, there is a positive correlation between the inheritance of "A" and "B." On the other hand, if the change is such that the two chromosomes are aBC and AbC, there is a negative correlation between A and B. In other words, the determinants remain correlated in the same way they entered the combination. There may be breaks in these correlations, however, as Morgan has shown in *Drosophila;* and these breaks in correlation occur in a constant ratio. Diagrammatically, it may be said that A and B are always the same distance apart in the chromosome structure and that the determinants "cross over" from one member of a pair to the other every so often. All of the gametes in the first case are not ABC and abC, for example. Some of them will be AbC and aBC. And the same percentages of these cross overs are found in the second case where "A" and "B" are correlated negatively. Furthermore,

if C should become c, and the chromosome pair take the form ABC and abc, there are definite relations between the three determinants. Breaks in correlation occur, and this ratio is constant, so that if given the percentage of breaks of correlation between "A" and "C" and "B" and "C," the percentage of breaks between "A" and "B" can be predicted. If there is a break in the correlation between "A" and "C" 30 times in 100, and a break between "B" and "C" 10 times in 100, then there will be breaks in the correlation between "A" and "B" 20 times in 100.

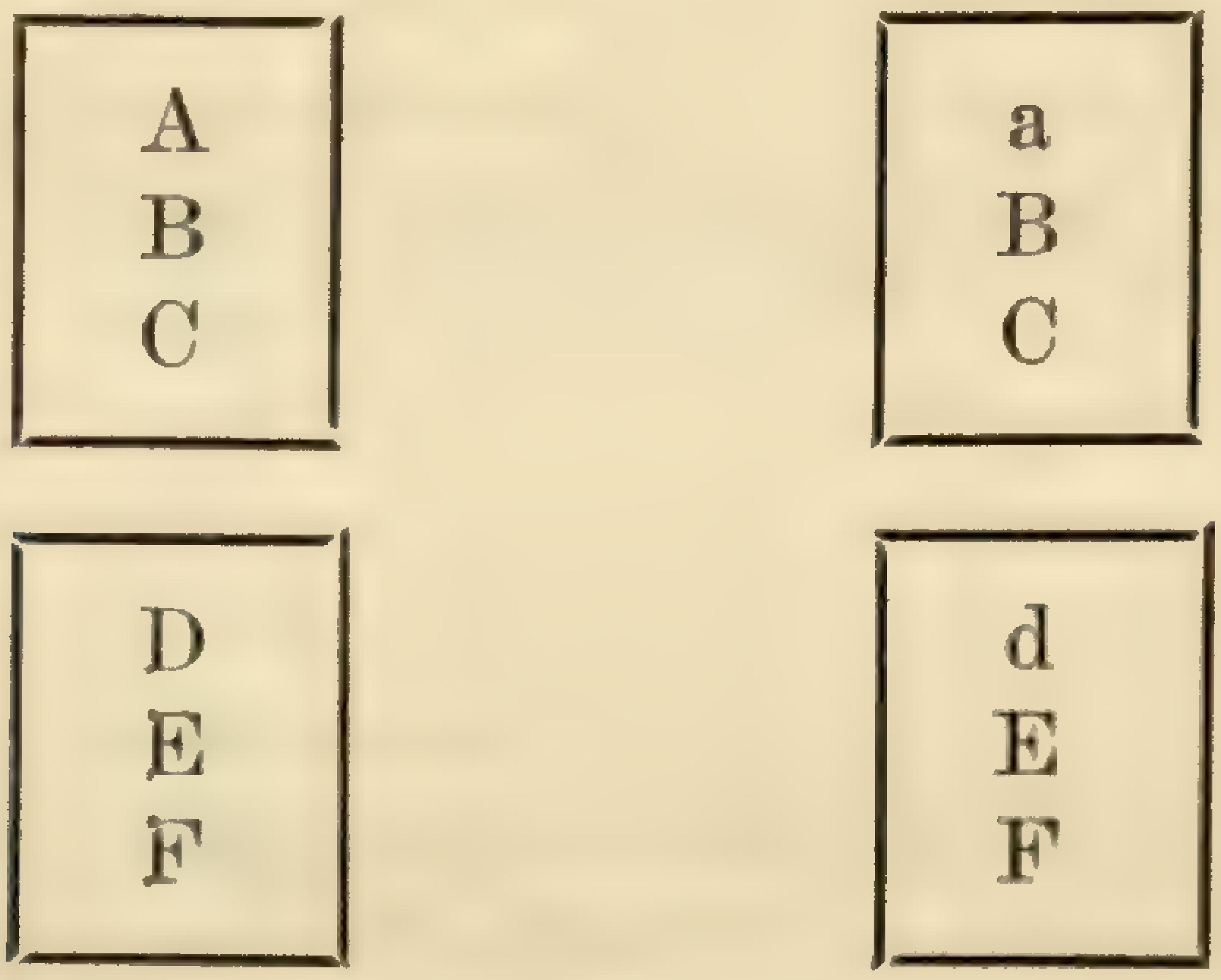

FIG. 5

Likewise, the determinants in the second pair of chromosomes are coupled together in their inheritance. D, E and F have each their peculiar linkage to the other, a linkage that remains comparatively constant. Yet the determinants in the second pair of chromosomes are entirely independent from those in the first pair in their inheritance. For example, if, as shown in Fig. 5, "A" should become "a" in either member of pair number one, and "D" should become "d" in either member of pair number two, Mendelian dihybridism would result. Furthermore, if "A" and "D" should each have the function of affecting the same general character complex in somewhat the same manner, there would be an apparent 15:1 ratio if dominance were complete or a series of types ranging from the type of one grandparent to that of the other, if dominance is lacking.

These are the main features that have been established

by recent work on hybrids. We have *pictured* them as actual chromosome functions, because every part of the description has been actual fact as far as the breeding experiments go. Our picture, it is true, is fictitious, for we do not know absolutely that the heredity mechanism is of this nature. But the facts do fit perfectly all that is known of chromosome behavior. It seems impossible, therefore, that there should be so many coincidences.

There are also two other pieces of evidence that fit in and round out the case. Bridges has shown that females occasionally occur in *Drosophila* bearing the sex-linked characters borne by the mother but showing no influence of those borne in the father. Such exceptional females were found to inherit directly from their mother the power of producing like exceptions, and it was proven cytologically *after the prediction had been made from the breeding facts* that these females resulted from the non-disjunction of the X chromosomes at the maturation of the eggs from which they came, and that one half of their daughters did in fact contain a Y chromosome in addition to two X chromosomes. This appears to be definite proof that sex-linked genes are borne by the X chromosomes.

The other important basis for regarding the chromosomes as the material basis for heredity also comes from Morgan's work on *Drosophila ampelophila*, this being the only species upon which sufficient work has been done to give a reasonable basis for the conclusion. *All of the hundred and thirty or so mutations in this species upon which Morgan and his students have worked are so linked together in heredity that they form four groups corresponding to the four pairs of chromosomes found in the species. If one single character should be found that did not fit into one of these four groups, the whole theory would break down. But no such character has appeared.*

This completes the case for the chromosomes as regards the main facts, and it seems only proper that a fair-minded jury of scientists should render verdict for the plaintiff. No case is so bad, however, that a lawyer can

find nothing to say for the defense and scientists in this respect resemble the men of the bar. Certainly there are some outlying facts, but they are comparatively unimportant. If a series of important facts should at any time be found which do not fit, the chromosome mechanism should be looked into. It is likely that the explanation will be found in an abnormal chromosome behavior as was the case in the aphis.

Practical Conclusions and Discussions

If now it be accepted as a reasonable premise that the chromosomes are the chief if not the sole bearers of hereditary determinants of body characters, and that their behavior is a rough indication of the mechanism of heredity; what cytological facts, if any, can be made useful at present or in the future to plant and animal breeders? If such data exist, they should be put to service; if it is likely that such facts can be found, investigations should be undertaken. The broad question may be divided into three parts which will be discussed in regular sequence:

1. What are the relations of chromosomes to somatic characters?

2. What are the relations of normal chromosome behavior to the transmission of characters?

3. What are the relations of peculiar or unusual chromosome behavior to the transmission of characters?

Relations of Chromosomes to Internal Characters

Some very interesting observations have been made on the relations of internal and external characters to chromosome number.

Farmer and Digby in a comparative study of the cells of a fern of the genus *Athyrium* with similar cells of three of its varieties, found that the measurements were successively larger in the three varieties than in the species, and that there was a corresponding increase in the number of chromosomes, the gametic numbers for the species and its varieties being estimated at 76–80, 84, 90 and 100,

respectively. Investigations on another fern, *Lastrea*, did not corroborate these results, however, in one variety the chromosomes being more numerous and the cells smaller than in the parent type.

Gates by comparing nuclei and cells of different tissues of *Œnothera Lamarckiana* and similar structures in its "mutant" *O. gigas* with double the number of chromosomes, found that the *O. gigas* cells and nuclei were always larger, varying from a comparative ratio of 1:1.5 to 1:3. At the same time, it would hardly be wise to maintain that this is always the case, for only a few individuals were investigated.

Primula sinensis has two forms in cultivation, similar except as to size. The giant form has flowers about one and one half times the size of those produced by the ordinary form. Gregory investigated these two forms cytologically to determine the cause of this increase. The nuclei and the chromosomes of the giant form were a little larger, though the difference was hardly a measurable one. The chromosome number was the same in both the forms. In a later investigation he has found that some exceedingly large plants with nuclei distinctly larger than those of the normal form had double the number of chromosomes normal to the species.

Boveri investigated this same relation of cells and nuclei to chromosome number in N, 2N and 4N larvæ of the sea urchin. From these studies, he concludes that chromatin is non-regulatory, and in the case of decrease, unregenerable, the cytoplasm in contrast showing the fullest regulatory activity. Further, the size of the larval cells is governed by the chromosome mass and the cell volume is directly proportional to the chromosome number. On the other hand, Conklin's investigations on annelids, mollusks and ascidians lead him to take a position opposed to that of Boveri. He says:

> The size of the nucleus, centrosomes and chromosomes is dependent upon the volume of the cytoplasm is clearly shown in *Crepidula,* where in large and small blastomeres, these structures are invariably proportional in size to the volume of cytoplasm.

Neither chromosomes nor nucleus control, the size of the cell in annelids, mollusks or ascidians.

Relations between Chromosomes and External Characters

Thus there seems to be no constant relationship even between nuclear or cell size and number of chromosomes, and bonds of union between external taxonomic characters and chromosome number seem to be still more tenuous. It is true that certain giant Primulas and Œnotheras had more chromosomes than were characteristic of the normal forms, but it is just as clear that all giant Primulas (and the same is probably true of Œnotheras, from the work of Heribert-Nilsson and of Geerts) do not have abnormal chromosome numbers.

Results on several species of both animals and plants are interesting in this connection.

The thread worm, *Ascaris megalocephala,* has two varieties, *bivalens* and *univalens,* the former having as a 2N number four chromosomes, the latter two chromosomes. Nothing is known as to the origin of these two forms. They are found parasitic in the same host individual and neither form is rare. According to Herla, they hybridize freely and produce embryos whose cells have three chromosomes, but no mature hybrids have ever been found. Meyer could distinguish no anatomical differences between the two varieties.

Rosenberg investigated the reproductive structures of two species of sundew and found one to have double the chromosome number of the other. A subsequent comparison of anatomical and taxonomic characters failed to show any sharply marked differences between them except in size. The form having the smaller chromosome number was smaller and less robust. They inhabit the same territory and produce natural hybrids which are sterile.

Rosa canina has two varieties which have the same taxonomic characters, but one form has thirty-four while the

other has only sixteen chromosomes. The form with thirty-four chromosomes is apogamous and reproduces without fertilization, but that one must not conclude that apogamy is necessarily associated with a double or an increased chromosome number, is clear from the case of *Rumex*. *Rumex* was investigated by Roth; one species, *R. cordifolius*, having forty chromosomes as its 2N number, required fertilization to produce offspring; another species, with only sixteen chromosomes, was apogamous.

A short list of nearly related species or species with two varieties varying in their chromosome numbers with their character differences, if present, is given below.

Name	Date	N	2N	Characters	Investigator
Alchemilla Eualchemilla . . .	1904	32	64	Apogamous	Strasburger, E.
" *aphanes*	1904	16	32		" "
Ascaris megalocephala	1883	2	4	Alike externally	Van Beneden
" "	1895	2	4		Meyer, O.
" "		1	2		" " and others
Ascaris lumbricoides	1887		24		Boveri, T.
" "	1887		48		" "
Dahlia variabilis	1911	16	32		Ishikawa, M.
" "	1911	32	64		" "
Drosera rotundifolia	1909	10	20		Rosenberg, O.
" *longifolia*	1909	20	40	More robust, etc.	" "
Echinus microtuberculatus .	1888	9	18		Boveri, T.
" " .	1902	18	36		" "
Helix pomatia	1903	24	48	Alike externally	Ancel, P.
" "	1896	12	24		v. Rath, O.
Nephrodium molle	1908	64	128	None mentioned	Yamanouchi, S.
" "	1908	66	132		" "
Œnothera lamarckiana	1911	7	14		Gates, R. R.
" *gigas* form	1909	14	28	Large and coarser	" " "
Primula sinensis	1909	12	24		Gregory, R. P.
" giant form	1909	12	24	More robust	" " "
" " "	1914	24	48	" "	" " "
Rosa canina	1909		34	Apogamous	Rosenberg, O.
" "	1904	8	16		Strasburger, E.
Thalictrum minus	1909	12	24		Overton, J. B.
" *purpurascens* . . .	1909	24	48	Apogamous	" " "
Zea Mays, "White Flint".	1911	10			Kuwada, Y. "
" " , "Sugar"	1911	12			"

What conclusions can be drawn from these facts? Certain botanists have attempted to connect chromosome doubling with apogamy, as usually the chromosome number in apogamous species is higher than in the normal species of the same genus; but there is no evidence of

apogamy in *Œnothera gigas,* and in *Rumex* the form with the low number of chromosomes is apogamous while the form with the high chromosome number requires fertilization. On account of these exceptions, therefore, it seems probable that the cause of apogamy is deeper than a mere doubling of the chromosomes, even though doubling may usually accompany such a change in reproductive habits.

Variation in chromosome number in the same species has been proposed as a cause of general variation in somatic characters, but the evidence is not clearly in favor of such a theory. In the fern *Nephrodium molle* Yamanouchi found spermatid cells to be of two sorts, those with sixty-six and those with sixty-four chromosomes. This would mean that *Nephrodium* has two gametophyte forms and two sporophyte forms, externally identical, so far as our present knowledge goes, but differing in their chromosome numbers.

Further, sporophytes developing from the prothallia of ferns without the intervention of a sexual process have the N instead of the 2N chromosome number, yet apogamously developed fern sporophytes, except as to chromosome number, are indistinguishable from normal sexually produced individuals of the same species.

Many writers have been tempted to postulate a causal relation between the numerical variation of chromosomes among the species of a genus and the genera of a family and their specific and generic characters. The thirty or more species of Compositæ investigated have shown a remarkable variation in their chromosome numbers, the 2N numbers ranging between six and sixty, and, as is well known, the Compositæ possess an infinite variety of sharply contrasting characters. But the lily family also has an enormous number of characters in its species and genera, and the genus *Lilium,* with its great variety of characters distributed among forty-five species, is typical of the other genera of the family, as far as present investigations go, in having the same chromosome number for

all of its species. Others suggest that the more chromosomes a plant species possesses the greater is its variability. Thus Spillman[3] speaks of the low variability of rye, suggesting its small chromosome number (six or eight) as a possible reason; for maize, having probably from twenty to twenty-four chromosomes, is infinitely more variable than rye. However, Britton's "Manual" selects *Crepis virens* for special mention as an extremely variable species from among the four or five other species listed under that genus, and it is known that *C. virens* has only six chromosomes, while three other species of *Crepis* investigated all have higher numbers. Again, according to Wiegand, the *Canna* has only six chromosomes, yet every gardener is well acquainted with the infinite variety in Cannas.

The Chromosomes and Variability

After a consideration of the above facts, one may well hesitate to state that there is even a high degree of correlation either between variability in chromosome number and general variability, or between high numbers of chromosomes and a high degree of variability in specific characters. On the other hand, it is not certain that the data upon which our discussion is based are relevant to the case in hand. We have discussed a possible relationship between chromosome numbers and species complexity and variability as found in the wild. This is not at all the same thing as discussing the relationship between chromosome number and true variability. It is true that complexity and specialization of plants and animals seem to have no connection with chromosome number, and that within a family a genus or a species profusion of taxonomic characters do not go hand-in-hand with high chromosome numbers. But in these cases our data come from persistent forms. What the actual inherent variability of the protoplasm is in most cases we do not know. *Drosophila ampelophila*, a species with only four chromo-

[3] Six according to Westgate's unpublished data; eight according to Nakao.

some pairs, is considered to be very constant in its characters from the taxonomist's standpoint, yet by careful continued observation Morgan has succeeded in detecting over 130 mutations.

From a strictly mathematical standpoint, it would seem that if other things are equal, variability would take place in proportion to the number of chromosome units. The difficulty is that in no case do we know anything whatever about the relative complexity of any particular chromosome unit. One must infer, however, that the 47–48 chromosomes in man are individually much more complex than the 128–132 chromosomes in the fern *Nephrodium molle.* If this inference be correct there are reasons why alteration in determinants may occur in direct proportion to the number of chromosomes or rather to the mass of chromatin without there being visible somatic variability in the same ratio. Let us construct an imaginary plan for preventing visible variation without preventing change in chromosome determinants. Unquestionably the simplest means is to double the chromosome number. Selecting, for example, a species with four chromosomes, let us suppose that fertilization occurs without a reduction division at some time or other. Then instead of a dual organism with two sets of chromosomes of similar function, one from the male and one from the female parent, there would be a quadruple organism with two sets of similar chromosomes from each parent. Any germinal change which would produce a *new dominant* character would be apparent immediately, but for a recessive change to appear—and these are many times as numerous as the others—it would be necessary to have identical changes occur in two chromosomes. Following out this line of reasoning, it is not hard to see what a great possibility for uniformity there is in further chromosome duplication, provided the actual fact of duplication makes no great change in the organism. That chromosome doubling has no decided visible effect we have seen from the cases already described; and since so many nearly related spe-

cies and varieties have their chromosome numbers in series 1:2:3:4, etc., it seems by no means improbable that what we have imagined above has actually occurred many times. And if one may believe that the event has the result supposed, all the worry about relationships between chromosome number and height of species in the scale of evolution may be eliminated.

Normal Chromosome Behavior and Heredity

The second query, concerning the relation of normal chromosome behavior to the transmission of characters, is much more important than the one just examined, but it can be discussed more briefly. By normal "chromosome behavior" is meant a reduction division where maternal and paternal chromosomes approach each other in definite pairs (if homologous pairs are present), chance only governing the passage of either to a particular daughter cell. This is probably the usual behavior in the higher plants and animals, and upon this behavior depends Mendelian heredity in the narrow sense. The thesis to be submitted and scrutinized is the following: *The maximum possible difficulty in the improvement of animals and plants by hybridization usually depends directly upon the chromosome number.*

When a mutation in a single determinant takes place in the germ cells of a plant, such as may cause the loss of red color in the corolla, crosses between such a form and the normal give a monohybrid Mendelian result. Two mutations in non-homologous chromosomes gives in a similar way a dihybrid result. Such simple conditions, however, are not met with very frequently. For example, White found that a fasciated tobacco when crossed with the type from which it sprang and from which it probably differed only by this single determinant, gave a monohybrid Mendelian ratio in the F_2 generation; but when the fasciated type was crossed with other types the result was a complex F_2 population. This population was susceptible of analysis, nevertheless, and showed that the various

varieties with which the fasciated type was crossed differed from it by several determinants, each of which was transmitted independently *though they every one affected the development of fasciation.* This illustration is not one of a rare phenomenon. It is what geneticists find constantly in their experiments. Presence or absence of a particular character may depend upon the presence or absence of a particular essential determinant, but, given this determinant, sooner or later the investigator finds several other determinants which modify the expression of the character. The existence of these modifiers has been the cause of a great deal of confusion in the analysis of breeding results, but in reality the inheritance is simple. The experience that all investigators who have worked intensively have had with them shows that practically all somatic characters are due to multiple determinants in the germ cells. It merely depends on the relative difference between the germ plasms brought together in crosses, how complex the resulting F_2 populations appear. Since even apparently simple characters are thus due to complex germinal interactions, that results of crosses made for the purpose of improving such intangible things as yield, size, quality, etc., should be complex, is not astonishing. In the comparatively extensive experience that the writer has had in breeding tobacco, maize, peas and beans the wide variability of the F_2 population in crosses between distinct varieties leads him to think that it is extremely common for such varieties to differ qualitatively in *every chromosome.* Furthermore, the relative complexity of the segregating populations is much greater in tobacco than in corn and greater in corn than in peas or beans. What can this mean but that when varieties are found that differ qualitatively in all of their chromosomes, the complexity of the result varies directly with the number of chromosomes present.

In Mendelian inheritance the number of actual types (both homozygous and heterozygous) present in the F_2 population when all are represented is 3^n, and the number

of individuals that must be present to give an equal chance for the presence or absence of an individual of every type is 4^n, where n represents the number of allelomorphic pairs. This being true, if differences in all of the chromosomes are predicated in tobacco and in pea crosses, the maximum number of individuals necessary in the F_2 generation to allow for one reproduction of each of the grandparental forms is 4^{24} in the first case and 4^7 in the second case. It is clear that there is an absolutely overwhelming difference in the difficulty of recovering the grandparental forms in the two examples.

Now this is about what one wishes to do in many plant-breeding problems. It is desired to combine one or two characters from one parent with all of the other qualities of the second parent. And such has been my experience that I believe that this maximum possible difficulty in the operation as predicated by qualitative differences in all of the chromosomes often occurs. There can be no question on these grounds of the importance of determining the number of chromosomes in a species before beginning a complex plant-breeding problem, and thus being able to comprehend the maximum possible difficulties that may be encountered. How greatly these difficulties vary may be seen in the very incomplete list of chromosome counts in common plants that is given below.

Common Name	Scientific Name	N	2N	Date	Investigator
Banana	*Musa sapientum*, "dole"	8	"16"	1910	Tischler, G.
"	*Musa sapientum*, "Radjah Siam"	16	"32"	1910	" "
"	*Musa sapientum*, "Kladi"	24	48	1910	" "
Bean	*Phaseolus vulgaris*	8	16	1904	Wager, H.
Calla lily	*Richardia Africana*	8	16	1909	Overton, J. B.
Canna	*Canna indica*	3	6	1900	Wiegand, K. M.
"	" "	8	more than 10	1904	Körnicke, M.
Corn	*Zea Mays*, "yellow starchy" "amber rice pop," "black starchy," "golden broach field," "white flint"	10	"20"	1911	Kuwada, Y.
"	*Zea Mays*, "red starchy," "red sugar"	9–10	"18–20"	1911	" "
"	*Zea Mays*, early 8-rowed sugar	9–12		1911	" "

Common Name	Scientific Name	N	2N	Date	Investigator
"	*Zea Mays*, early sugar...	12		1911	" "
Cotton......	*Gossypium*," hybrid"...	28	"56"	1903	Cannon, W. A.
"	" , "Egyptian".	20		1910	Balls, W. L.
Currant.....	*Ribes* 2 sp.............	8	"16"	1906	Tischler, G.
Dandelion...	*Taraxacum confertum*...	8	"16"	1909	Rosenberg, O.
" ...	" sp..........	12or13	about 26	1905	Juel, H. O.
Elderberry...	*Sambucus* sp...........	18	38	1909	Lagerberg, T.
Evening primrose ..	*Œnothera grandiflora*....	7	14	1909	Davis, B. M.
Evening primrose...	*O. lamarckiana*........	"7"	14	1907	Geerts, J. M.
		7		1911	Gates, R. R.
Evening primrose...	*O. gigas*...............	14	28	1909	Gates, R. R.
Fern........	*Nephrodium molle*......	64 or 66	128 or 132	1908	Yamanouchi, S.
Flag........	*Iris squalens*...........	12	24	1900	Strasburger, E.
Hawksbeard.	*Crepis lanceolata* var. *platyphylum*.........	5	10	1911	Tahara, M., and M. Ishikawa.
" .	*Crepis virens*...........	3	6	1909	Rosenberg, O.
" .	*Crepis tectorum*........	4	8	1905	Juel, H. O.
" .	*Crepis japonica*........	8	16	1910	Tahara, M.
Lily........	*Lilium martagon*.......	12	24	1884	Guignard, L.
Lily-of-the-Valley....	*Convallaria majalis* ...	18	"36"	1899	Wiegand, K. M.
Lily-of-the-Valley....	*Convallaria majalis*.....	16	32	1909	Sauer, L. W.
Mulberry....	*Morus alba*, "Shirowase"	17?	40–50	1910	Tahara, M.
"	*Morus indica*..........	14	28	1910	Tahara, M.
Nightshade..	*Solanum nigrum*.......	36	72?	1909	Winkler, Hans
Onion.......	*Allium Cepa*..........	"8"	"16"	1898	Schaffner, J. H.
Peony.......	*Paeonia spectabilis*.....	12	"24"	1893	Overton, E.
Pea.........	*Pisum sativum*.........	7	14	1903	Cannon, W. A.
Persimmon..	*Diospyros virginiana*....		30 or more	1911	Hague, Stella M.
Pine........	*Pinus laricio*..........	12	24	1899	Chamberlain, C. J.
Rice........	*Oryza sativa*...........	12	24[4]	1910	Kuwada, Y.
Rose........	*Rosa* sp.—3 species.....	8	16	1904	Strasburger, E.
Tobacco.....	*Nicotiana* sp...........	24	48	1913	White, O. E.
Tomato.....	*Solanum lycopersicum*...	12	24	1909	Winkler, Hans
Tulip.......	*Tulipa Gesneriana*......	12	24	1901	Ernst, A.
Wake-robin..	*Trillium grandiflorum*...	6	12	1899	Atkinson, G. F.
Wheat......	*Triticum vulgare*.......	8	16	1896	Körnicke, M.
"	" "	8	"16"	1893	Overton, E.
"	" "	8	"16"	1908	Dudley, A. H.

Among these figures are found four of our most important crops—wheat, tobacco, corn and cotton. They contrast strikingly in their chromosome numbers. Wheat and tobacco, species in which the flowers are naturally self-pollinated, have 8 and 24 chromosomes, respectively,

[4] "But we often find a larger number." Quotation marks refer to inferred numbers rather than actual countings.

in their gametes. Corn and cotton, species usually cross-pollinated, have 10–12 and 20–28 chromosomes, respectively, in their germ cells. These species all have been under cultivation since before there has been recorded history. Many varieties of each exist. It is not at all improbable that with thousands of years of cultivation and selection under diverse conditions, mutations in most of their chromosomes have persisted. If, then, *improvement* means working on character complexes that involve almost all of the plant functions, it does not seem improbable that the actual difference in the difficulty of improving wheat and tobacco is as $4^8:4^{24}$, or about 1 to 4,295,000,000. In like manner corn and cotton compare in the ratio $4^{10}:4^{28}$, or 1 to 68,720,000,000. And is it not true that modern improvement in most of these crops does involve nearly all the plant functions? Yield in wheat involves number and size of grain, and number of culms, with all that these things include in plant economy; yield of tobacco involves number, size and thickness of the leaves. Quality, a mystical word, is perhaps still more complex. In wheat, it takes in habit of growth of both root and stem and such other characters as go to make up strength and hardiness, thickness of pericarp, size of aleurone cells, and the physical and the chemical character of both endosperm and embryo, as well as their size ratios in regard to each other. In tobacco, it includes thickness and strength of leaf, color, texture and all chemical and physical characters that make for flavor and "burn."

One may say that this is all very well as a theory, but that it is all theory, and ask what support is given to it by practise. I have had personal experience with but two of these four crops. I have worked extensively and intensively with corn and tobacco for some ten years. But I have followed carefully the published experiments in breeding wheat and cotton and have seen several of the more important experiments. *And I may say that it was my observation of the extreme difficulty in the experiments with cotton and tobacco as compared with corn and wheat that led to this theory of the cause.*

In proposing this thesis, the chromosomes have been considered as pairs of freight boats loaded with character determiners, shifted bodily to the daughter cells by internal forces of which we are ignorant. Yet this is not the whole truth. The determiners in particular chromosomes seem to be tied together more or less tightly, but they are not always transferred as one package. They are coupled in their transmission to the next generation, but this coupling is not perfect. Breaks in the coupling occur and there is order and regularity in these breaks. Our knowledge on these matters rests upon the researches of Morgan on *Drosophila,* Bateson on the sweet pea, and Tanaka on the silkworm, so it is not certain whether these are common grounds for this regularity or whether each species has regular laws which control the breaks in correlation. But in either case, these breaks do not interfere with our proposition. They only complicate matters. In most of the cases in *Drosophila,* where they are best known, linkage is comparatively tight, *i. e.,* breaks are somewhat rare; but they may become so frequent as to simulate inheritance from separate chromosomes. In those cases our theory is of no value, but if *Drosophila* is any criterion by which to judge, such conditions are very unusual.

Abnormal Chromosome Behavior and Heredity

The third query concerning the relations of peculiar or unusual chromosome behavior to the transmission of characters may be passed over with a word. In certain insects, for example, bees, wasps, aphids, phylloxerans, etc., odd sex ratios and attendant complexities have long been known. These have been cleared up more or less completely by cytological studies. They depended upon chromosome behaviors that are not usual in animals or plants. Similar peculiar chromosome mechanisms may be present in many other species. Attention is merely called to the fact that if experiments on any plant species appear to show that its characters do not obey the laws that have been demonstrated for so many types, their

cytological eccentricities should be looked into. In them will probably be found the key to the situation. The Œnotheras may be mentioned as a case in point. Their heredity in many cases is not what would be expected by analogy with other plants. We know that in some ways the behavior of their chromosomes is irregular. Further study will probably show that this is the sole cause of their anomalous heredity.

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REGENERATION POSTERIORLY IN ENCHYTRÆUS ALBIDUS[1]

H. R. HUNT

THE primary object of the following experiments was to determine whether *Enchytræus albidus* can regenerate posteriorly, when cut at regions of the body varying from near the posterior end to near the anterior end. Secondly, an attempt was made to compare the rates of regeneration per day posteriorly at the different levels at which the worms were cut in two.

No experiments have been published in which the capacity of this species to regenerate posteriorly has been tested. Nusbaum ('02; '04) studied the histological processes in the regeneration of the Enchytræidæ anteriorly and posteriorly. He found that regeneration anteriorly does not take place as readily as regeneration posteriorly, and that never more than two or three segments regenerate anteriorly.

The animals used in the present experiments were collected in abundance from the coarse gravel of the tidal zone on the seashore at Cold Spring Harbor, Long Island, New York. Six sets of experiments were conducted. Each of the worms was cut into two pieces, the anterior and the posterior pieces being preserved. The average number of segments in this species is not far from sixty. The regions selected for cutting were such as to give fairly comprehensive data as to the regenerative capacity posteriorly at different levels. In the first set of experiments the worms were so cut as to leave only about eight anterior segments; in the second set about sixteen anterior segments; and in the third set about twenty anterior segments. In the fourth set the cut was made near the middle of the worm; in the fifth about sixteen posterior

[1] Contributions from the Zoological Laboratory of the Museum of Comparative Zoology at Harvard College, No. 260.

segments were removed; and in the sixth eight posterior segments. The worms were anesthetized with chloretone, and the operation was performed under a dissecting microscope. The pieces were placed in small sterilized glass bottles, each containing a strip of filter paper and enough sterilized sea water to keep the animals well moistened. Ten pieces of approximately the same length were kept in a single bottle. Throughout the experiment the bottles, each one stoppered with a cork, were kept in an ice chest to restrict the growth of bacteria. The work was begun early in July, 1913, and was continued until the first of October. At the middle of August it became necessary to carry away from the seashore the material then living. After this, fresh water was used for moistening the worms and cleaning out the bottles. The worms, however, seemed to regenerate as well in the fresh-water as in the salt-water environment. The analysis of the results of the experiments was done in the zoological laboratory of Harvard University.

It was found that the *length* of the regenerated segments, as compared with that of the segments in the adjacent unregenerated part of the worm, was a fairly accurate criterion for determining the number of regenerated segments. To test the accuracy of this criterion, parts of eight worms consisting of the twenty most anterior segments were allowed to regenerate for about eight weeks. Having taken the precaution to determine accurately the number of segments in each of the pieces at the time of the operation, it was easy to determine how many segments had regenerated, for of the total number of segments at the end of the experiment all except the original twenty were, of course, regenerated segments. The result thus obtained was compared in each worm with that obtained by counting in the same worm the number of segments posterior to the point where there was an abrupt change in the length of the segments, that point indicating the region of the cut. Table I gives the data for this comparison. The results show that the method which

was used to determine the number of regenerated segments is accurate to within one or two segments, for it will be noted that the results by the two methods never differ by more than two segments, usually by only one. The worm's body is so short that it was found impracticable to secure exactly eight, sixteen, etc., segments in every piece used in the whole series of experiments.

TABLE I

	Number of Segments Regenerated	
Number of the Worm	Total Number Minus 20	As Determined by Segment Length
1	6	7
2	18	18
3	20	22
4	23	24
5	21	22
6	15	14
7	18	17
8	10	12

The results obtained in each of the six sets of experiments have been condensed, for convenience, and are shown in Table II. In the first vertical column of this table the Roman numerals designate the number of the set of experiments. The horizontal lines corresponding to each of these sets give in succession, (1) the number of segments in the pieces used in the experiments, (2) the number of worms operated on, (3) the number that survived long enough to be observed, (4) the per cent of worms that survived and were observed, (5) the period during which the regeneration took place, (6) the number of segments (0 to 24) regenerated by the surviving worms, (7) the average number of segments regenerated in each set of experiments, and (8) the mean rate of regeneration per day of the worms in each set expressed in segments. This mean rate of regeneration was obtained by first computing the rate of regeneration per day (in segments) for each worm in the set, and then averaging all the results. In some worms the number of segments regenerated was observed twice, several weeks elapsing be-

TABLE II

No. of the Set of Experi-ments	No. of Seg-ments Re-moved[1]	No. of Worms Oper-ated	No. of Worms Ob-served	Per-centage Surviv-ing	Period of Observation	Number of Individuals Regenerating from 0 to 24 Segments Each																									Average No. of Segments	Rate per Day in Segments
						0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
I	52	95	6	6	36 days	..	..	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	5.0	.146
					57 "	..	..	..	..	..	..	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	9.0	
II	44	129	31	24	21–29 "	6	1	2	..	2	..	..	1	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2.9	.207
					34–35 "	..	..	..	..	..	2	..	1	2	3	2	1	1	..	1	1	..	..	..	..	..	..	..	..	..	9.4	
					60–81 "	..	..	..	..	..	..	..	1	1	..	..	1	..	..	..	..	3	..	..	1	..	1	2	..	..	15.8	
III	40	30	10	33	44–59 "	..	..	..	..	..	..	2	1	..	..	..	..	1	..	1	..	..	1	1	..	..	..	2	..	1	14.8	.267
IV	30	110	44	40	33–36 "	1	..	..	..	..	1	4	2	7	5	2	1	1	1	1	1	..	..	..	..	..	..	..	..	..	8.8	.238
					41–49 "	..	..	..	..	..	2	..	..	3	3	1	..	1	1	1	..	..	..	..	..	..	..	..	..	..	9.2	
					72–83 "	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	3	..	2	5	1	1	2	5	..	..	18.7	
V	16	164	51	31	26–31 "	5	3	3	..	6	4	4	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3.2	.129
					40–48 "	..	1	1	..	1	3	2	5	2	1	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	6.4	
					64–77 "	3	..	..	..	..	..	..	..	1	3	1	1	1	..	..	1	1	..	..	1	..	..	..	..	..	11.8	
VI	8	100	18	18	25–31 "	..	4	1	2	2	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2.8	.084
					62–79 "	..	3	1	..	..	..	1	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3.7	
Total.....		628	160																													

[1] The average number of segments in the body is about sixty.

tween the two observations, so that the total number of observations recorded in any one set of experiments may be larger than the number of worms observed in the same set.

The results of these experiments are summarized in the graph shown in Fig. 1, where the rates of regeneration per day (expressed in hundredths of the length of one segment) are measured on the axis of ordinates (Y), and the length (in number of segments) of the pieces that produced the regenerated parts, are measured on the axis of abscissæ (X). Since sixty is about the average number of segments in this species, that is the value which has been used in plotting the curve. A mathematical analysis of the rates of regeneration at the different levels shows that the difference in the mean rates of regeneration at any two successive levels is significant. But the temperature of the worms was not carefully controlled, and the periods during which the wounds were healing and the worms preparing to form new segments were included in the computation of the mean rates of regeneration. Therefore, the ratio between the rates of regeneration, as here computed, at any two of the six levels only approximates the ratio which would have been obtained between the rates at these two levels by subjecting all the worms to the same temperature conditions and by using in the computation of the mean rates of regeneration only the periods during which the segments were being formed. The curve *suggests,* however, that the rate of regeneration for the posterior half of the body is proportional, or nearly so, to the number of segments removed. Anterior to the twentieth segment the rate of regeneration decreases. May we not have here a curve depending on two opposing sets of factors; one which tends to increase the rate of regeneration as more segments are removed, the other to decrease the rate? In the latter set of factors the amount of available building material may be the most important element.

The worms seemed to regenerate equally well in a fresh-water or in a salt-water environment. Thirty-one of the one hundred and sixty surviving worms lived for about forty days in a fresh-water environment and regenerated. Twenty-six worms from which the sixteen posterior seg-

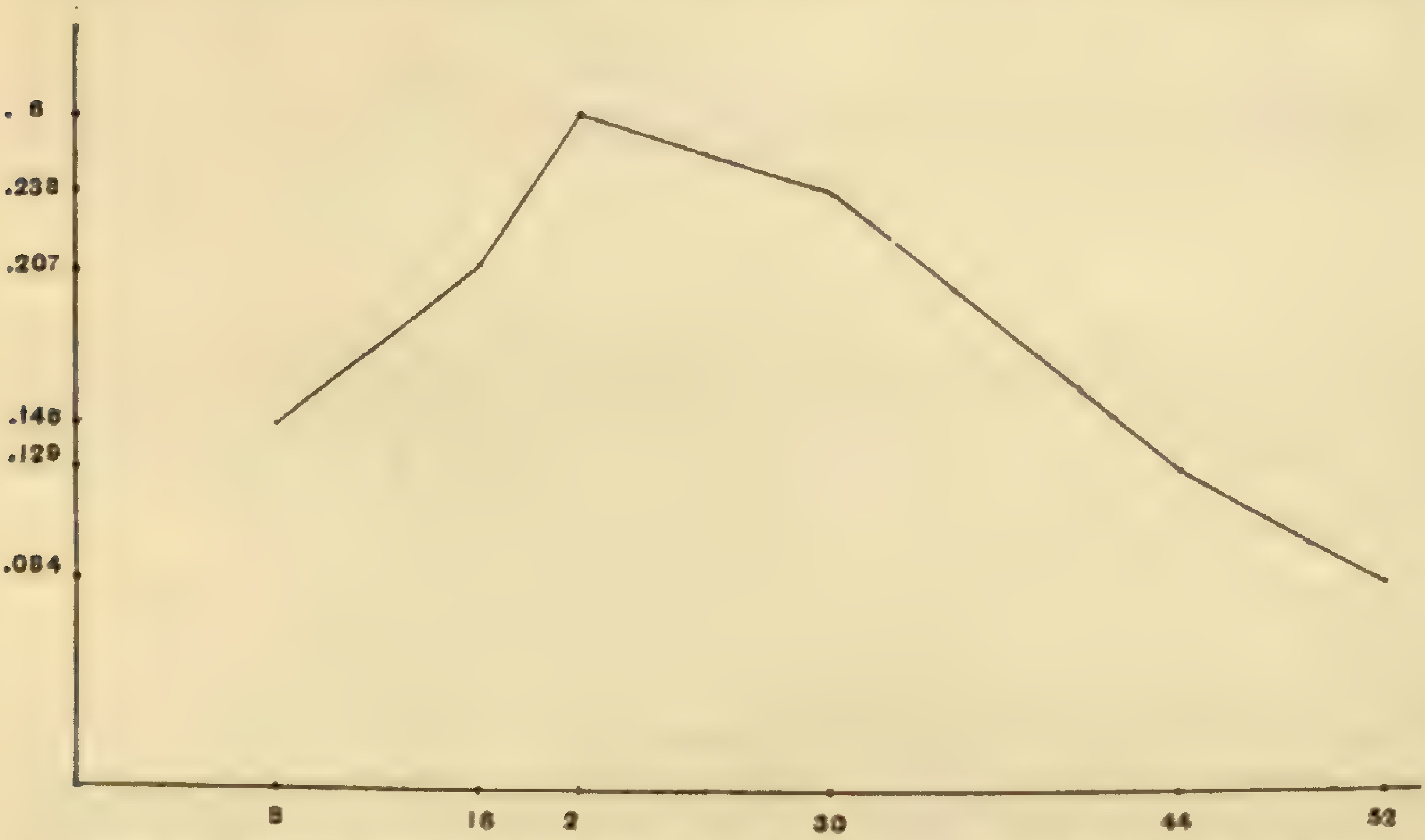

FIG. 1. Curve showing the daily rate of regeneration by pieces of six different lengths. The unit selected to measure the mean rate of regeneration at each of the six levels was 1/100 of a segment, while that used to measure the lengths of the pieces which produced the regenerated segments was *one* segment. In plotting the curve the length selected to represent 1/100 of a regenerated segment (on axis Y) was the same as that chosen to represent one segment (axis of X) of the pieces producing the regenerated parts.

ments had been removed, and twenty-six others from which the posterior half had been removed, regenerated almost contemporaneously for about thirty days in the same ice chest, and in a salt-water environment. Later in the season in a different ice chest eighteen worms from which the sixteen posterior segments had been removed, and thirteen from which the posterior halves had been removed, regenerated contemporaneously for about forty days in a fresh-water environment. When the sixteen posterior segments were removed the rate of regeneration in the salt-water environment was 0.02 segments per day *less* than in the fresh-water environment, while when the posterior halves were removed the rate of regeneration in the salt-water was 0.07 segments per day *greater* than in

the fresh-water surroundings. These facts show that the worms regenerate in both fresh and salt water. This is not surprising, since individuals of this species are normally found both on the seashore, where they live in a salt-water environment, and also in earth moistened with fresh water. Furthermore, with the exception of the sa-

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FIG. 2. Camera lucida drawing of the posterior end of a normal worm. Magnified 17 diameters.

FIG. 3. Camera lucida drawing showing the regenerated posterior end of a worm. The region posterior to *X* is regenerated. Magnified 17 diameters.

FIG. 4. Sketch of a regenerated double tail. Magnified about 17 diameters.

linity of the water used to moisten the worms, the worms which regenerated in the fresh-water surroundings were probably subjected to about the same conditions as those which regenerated in the salt-water. Therefore, the salinity of the water in the environment does not seem to affect the rate of regeneration. The data used in plotting

the curve shown in Fig. 1 were secured from worms which regenerated in the fresh-water, as well as from those which regenerated in the salt-water, environment. The above observations make it seem probable, therefore, that the form of the curve does not differ fundamentally from the form which it would have had if all the worms had regenerated in salt-water surroundings.

In. Fig. 2 is shown the normal appearance of the ventral aspect of the posterior end of a worm in which there has been no regeneration. It will be noticed that the length of the segments gradually decreases toward the posterior end; but in Fig. 3, which is a camera lucida drawing of the posterior portion of one of the regenerated worms, the length of the segments decreases abruptly at the point *X*, showing that to be the point at which the tail was removed.

Three worms from which eight posterior segments were removed regenerated double tails. Morgan ('97) and Michel ('98) observed the same phenomena in *Allolobophora fœtida*. One of these worms is shown in Fig. 4.

Some attempts were made to determine the rate of regeneration anteriorly at different levels on the worm's body. At present all that can be said is that regeneration posteriorly takes place much more frequently and rapidly than anteriorly.

The conclusions that follow from these experiments are:

1. *Enchytræus albidus* regenerates posteriorly when cut off at any level between eight segments from the posterior end of the body and eight segments from the anterior end. It will be noticed that although the mortality in pieces containing only the eight most anterior segments was about 94 per cent., yet those that did survive regenerated from three to eleven (on the average seven) segments. In other words, a piece from the extreme anterior end, containing only one eighth the number of segments in the whole worm, can regenerate nearly as many segments, on the average, as it had at the beginning of the experiment. Morgan ('97) found that in *Allolobophora*

fœtida anterior pieces of less than thirteen segments rarely, if ever, regenerate posteriorly. In *Enchytræus* the anterior limit of the capacity to regenerate posteriorly was not found.

2. The rate of regeneration seems to increase from the posterior end of the worm up to its middle almost in direct proportion to the number of segments removed. Anterior to about the twentieth segment the rate decreases.

3. Regeneration can take place either in a fresh-water or in a salt-water environment. Also, the salinity of the water seems to have little or no effect upon the rate of regeneration.

4. Double tails can be regenerated when the eight most posterior segments are removed.

5. Regeneration posteriorly takes place more readily than it does anteriorly.

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THE ORIGIN OF BILATERALITY IN VERTEBRATES[1]

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Many attempts have been made to determine how early in development the vertebrate egg becomes bilaterally symmetrical. The conclusions have been as varied as the attempts.

Before the subject can be discussed it is necessary to consider two fundamental propositions. The first is that there exists an active pole in the egg, and the second is that the anterior end of the embryo develops in this region, or at least in the active hemisphere.

The active pole is indicated at an early period by certain phenomena, such as secretory activity, accelerated yolk metabolism, formation of pigment, position of nucleus, expulsion of polar bodies, etc. Hatschek says that "it is probable that a polar differentiation is present in the unfertilized ova of all the metazoa, through which the most active and least active poles can be determined." Whether or not Hatschek's statement be true, it is certain that if the area in which cleavage grooves first appear be traced backward a differentiation in this area can be found in a very early stage. We are thus enabled to speak of an active pole and an opposite inactive pole. A line passing through the two is designated as the primary ovic axis.

That the active pole or hemisphere gives rise to the embryo was first pointed out by Jan. Swammerdam in his "Bibel der Natur." This view was later supported by Prevost and Dumas, von Baer, Reichert, Cramer, Newport and others. Pflüger, however, believed that the greater portion of the embryo was formed from the inactive hemisphere and his view was supported by Roux, O. Hertwig and others. Most of the later investigators

[1] With observations by C. O. Whitman on Bufo.

including Morgan and Tsuda, Assheton, H. V. Wilson, King, Smith and others have generally agreed that the head end of the embryo forms from the active hemisphere and the caudal portion from the inactive. My own experiments on a considerable number of Amphibia have led to the conclusion that the head of the embryo forms from material which lies at, or near, the active pole of the egg. It thus seems fair to assume that the cephalic portion of the embryo is formed from the active hemisphere.

As stated there have been many attempts to determine how early in development the egg shows bilateral symmetry. Some claim bilateralism for the primitive ovum. Others hold that this condition is not present from the first, but originates at some later period. This period may precede or follow the deposition of the egg. Those who regard the egg as bilaterally symmetrical before deposition claim that this is manifested either through an excentric position of the egg nucleus, or an excentric pigmentation. Those who regard it as fixed after deposition are not in accord. By some the path of the spermatozoon is considered as the determining factor, by others the first or second cleavage groove, and by still others areas of accelerated segmentation.

The assumption that the egg is bilaterally symmetrical from the beginning is based upon nothing more than plausible hypothesis and naturally falls beyond the range of experimental proof.

Some (Schultze) hold that the excentric position of the egg nucleus together with the primary ovic axis determine bilaterality. The work by Roux, Jordan and others, shows that this is highly improbable.

Others (Roux, Morgan and Tsuda) maintain that the excentric arrangement of pigment enables one to determine bilaterality. Professor Whitman's observations which are recorded in a later paragraph, together with his drawings, indicate that the arrangement of the pigment is of significance in *Bufo*. The observations of Moskowski on *Rana*, Morgan's later observations on

Bufo, together with my own on *Amblystoma,* have thrown doubt upon this conclusion.

Still others (Newport, Roux) believe that the path of the entering spermatozoon and the primary ovic axis determine bilaterality. Jordan has shown that this view is untenable for *Diemyctylus.* Professor Whitman's observations, recorded in a later paragraph, show that this is not true in *Bufo.*

Thus each of these assumptions has been met by serious objections.

The idea that the first plane of cleavage determines the axis of the embryo was expressed as early as 1853 by Newport in the following words:

> I have long been aware that the axis of the embryo was in the line of the first cleft of the yolk.

From a series of experiments on the frog's egg Roux came to the conclusion that the first cleavage plane coincides with the median sagittal plane of the embryo. In the same year Pflüger reached the same conclusion. Supported by these eminent investigators the theory was very generally accepted. In working over the same field Rauber found that in the axolotl and frog the median plane of the embryo coincided with the second cleavage groove instead of the first. Shortly after the publication of Rauber's work, O. Hertwig working on the egg of *Triton* confirmed the observations of Rauber. In 1892 Roux modified his earlier view and stated that the second groove as well as the first often coincided with the median plane of the embryo.

In the following April the writer found from a series of puncture experiments on the egg of *Amblystoma* that exovates on opposite sides of the first cleavage groove were later found on one side of the embryo. The conclusion was that in these cases the first cleavage groove did not separate the right and left halves of the embryo.

In 1893 Jordan and the writer reviewed the experiments up to this date. We found that even in the descriptions and figures given by Newport, Roux, Rauber, there was evidence sufficient to show that the median

plane of the embryo often deviated widely from the first or second cleavage planes. We accordingly undertook an extended series of observations on the living segmenting eggs of *Amblystoma, Diemyctylus, Rana* and *Bufo*. Our conclusions were as follows:

> The first and second cleavage planes undergo, even in the earlier stages, extensive torsion. Everything indicates that the extent of this shifting increases greatly in later stages. This led us to conclude that the earlier cleavage planes and the embryonic axes have no vital connection and that the coincidence where it exists is of no fundamental significance.

The later observations by Grönroos, v. Ebner, Morgan and Tsuda, Kopsch and others have likewise emphasized the significance of these variations.

It is scarcely necessary to state that if these cleavage planes mark embryonic areas, the amount of material set apart in different eggs for similar parts of their respective embryos, must be exceedingly variable, and these excesses and deficiencies must be corrected by a corresponding retarded or accelerated growth until the norm is reached, but there is not the slightest evidence that such corrections occur.

These wide variations have been repeatedly observed not only in various amphibia but also in practically all classes of vertebrates: in *Amphioxus* by Wilson; in *Petromyson* by McClure, Kupffer, Eycleshymer; in Dipnoans by Semon; in Ganoids by Salensky, Dean, Whitman and Eycleshymer; in Teleosts by Coste, Hoffmann, His, Agassiz and Whitman, Kingsley and Conn, Clapp, Sobotta and others; in Reptiles by Agassiz and Clark, Oppel, Sarasin; in Aves by Coste, Koelliker, Kionka; in Mammals by Duval, v. Beneden, Assheton, Sobotta and many others.

The inevitable conclusion from such a mass of evidence can not be other than that neither the position or direction of cleavage grooves has the slightest significance as far as the setting apart of definite embryonic areas is concerned.

If then it may be considered an established fact that

neither the position nor the direction of the cleavage grooves enables one to predict the long axis of the embryo, we are naturally led to look for other phenomena which may be of significance. As stated in an earlier paragraph my experiments showed that the head end of the embryo is formed at, or very near, the active pole, and since this area is the one in which cell division is most rapid, it was concluded that the anterior end of the embryo, which is the first to differentiate, was indicated by this increased cellular activity. I accordingly stated that an area of increased cellular activity indicates the position of the head end of the embryo. As is well known, this area can be located with the advent of the first cleavage groove.

While the head end of the embryo may thus be readily located, the median plane of the body may lie in any one of an indefinite number of meridians. The question which now arises is which one of these meridians will represent the median plane of the future embryo.

The writer's studies on *Rana, Bufo, Acris, Amblystoma, Necturus* have shown that in another portion of the egg there is an area of smaller cells, and that this area of smaller cells always marked the region of the forthcoming blastopore. The blastopore in turn definitely fixes the posterior portion of the embryo.

With the recognition of these areas of accelerated cellular activity, the one at the active pole, indicating the position of the future head of the embryo, the other at the side of the egg, indicating the position of the forthcoming blastopore, it necessarily follows that the median plane of the embryo must coincide with a line passing through the centers of the two.

When these observations were first published in 1898, many questioned the existence of such a secondary area of cellular activity. Yet a search through the literature showed that such an area had been observed in many groups of vertebrates. Lwoff found such an area at the posterior end of the embryo of *Amphioxus*. The figures of the segmenting blastodiscs of Elasmobranchs, given

by Balfour, Rückert, Gerbe and Sobotta all show that in these forms such an area is present. In the Reptilia, Vay's studies on *Tropidonotus* show that an area of small cells represents the posterior end of the embryo. v. Koelliker first called attention to such an area in the blastodisc of the chick and suggested that it determines the position of the posterior end of the embryo. The later investigations of Duval and Kionka leave no doubt as to the frequent and probably constant appearance of this area in the locality which later becomes the posterior end of the embryo.

In 1904 the writer made a study of the egg of *Necturus*, which from its size is especially favorable for surface study. This work was undertaken with a view of ascertaining how early this secondary area could be located. It was found that as early as the fourth or fifth cleavage, the cells on one side began to divide more rapidly than any others, excepting those of the primary area. It was possible to predict in this form the median plane of the forthcoming embryo at an extremely early stage of cleavage.

The following year de Bussy from his studies on the Japanese *Cryptobranchus* emphasized the fact that he could find no secondary area of accelerated cell division such as had been described by the present writer. Yet Smith working on the American *Cryptobranchus* says that he finds "an accelerated cell division about a radius of the blastodisc which gives a condition of bilateral symmetry."

The writer felt that it was scarcely necessary to follow the subject further and should not have rehearsed the findings had it not been that certain material came into his hands last year which bears directly upon this subject. This material consists of unpublished descriptions and drawings made by the late Professor C. O. Whitman in June, 1894. These were turned over to me by the department of zoology of the University of Chicago. Professor Whitman's notes run as follows:

Hitherto we have obtained eggs the first week in June. This year we could find none until July 1. We had several night rains, enough to flood the low ground behind Breakwater Hotel. On the evening of June 30, the day after the rain fell copiously, the toads swarmed in this place, and had a carnival of noise; the whole place rang with so many voices as to be almost deafening. On the morning of July 1, we found a great many eggs. The following night the singing followed but much reduced, and only two pairs of toads were captured. The next night the water had gone except in one of the ditches and no toads were to be heard, and of course no eggs. It would seem that rains stimulate them to lay; and the lateness of the season may have been the reason that the egg-laying was confined almost entirely to a single night.

The unfertilized eggs are by some said to be unoriented, that is they are said to be unable to take the normal position assumed by the fertilized egg. The sperm is supposed by some observers to mark the first plane of division and to give the egg the power to right itself. I find that it is not true that the eggs will lie just as they happen to fall, although they do so more nearly before fertilization than after it. If an egg be separated from the rest and turned about for some moments with needles, so as to loosen its adhesion to the membrane, and then rolled to one side so that the equator is vertical, one observes that it slowly turns and in the course of a minute, or sooner, it takes the normal position with the blacker pole uppermost and the whiter showing a little on one side, when viewed from above. This was repeated several times and on several eggs with the same result. The motion is so slow that one does not notice it until after the lapse of some seconds.

I cannot affirm that all unfertilized eggs will right themselves; ordinarily they do not if left to themselves. They assume an irregular wrinkled appearance and have so little power of righting that they stick to the membrane enough to prevent it. When fertilized they contract and round up and get freedom of space to move in. The entrance of the sperm evidently increases the disproportions between the weights of the upper and lower pole. The upper pole becomes lighter and the egg rights itself more readily and quickly. The orientation of the egg is complete before fertilization.

In the eggs which are in the stage of first cleavage there is a small depression which I have found by examination of earlier stages is the "fovea germinativa" of Max Schultze, or the "fosette germinative" of Bambeke. I find further after fertilization, a second point or depression, which probably is the place of penetration of the spermatozoon. The fovea marks the upper pole, but is not placed at the middle of the upper hemisphere; it is excentric.

I followed two eggs which showed both the fovea and the spermatic dent. In neither did the first cleavage plane pass through this dent. In one case it passed far from it while the second cleavage passed near to it. In another case the dent is in the middle of one of the first four

cells, and on the darker side of the upper hemisphere. If this be the sperm track it does not determine the median plane of the embryo.

Cleavage

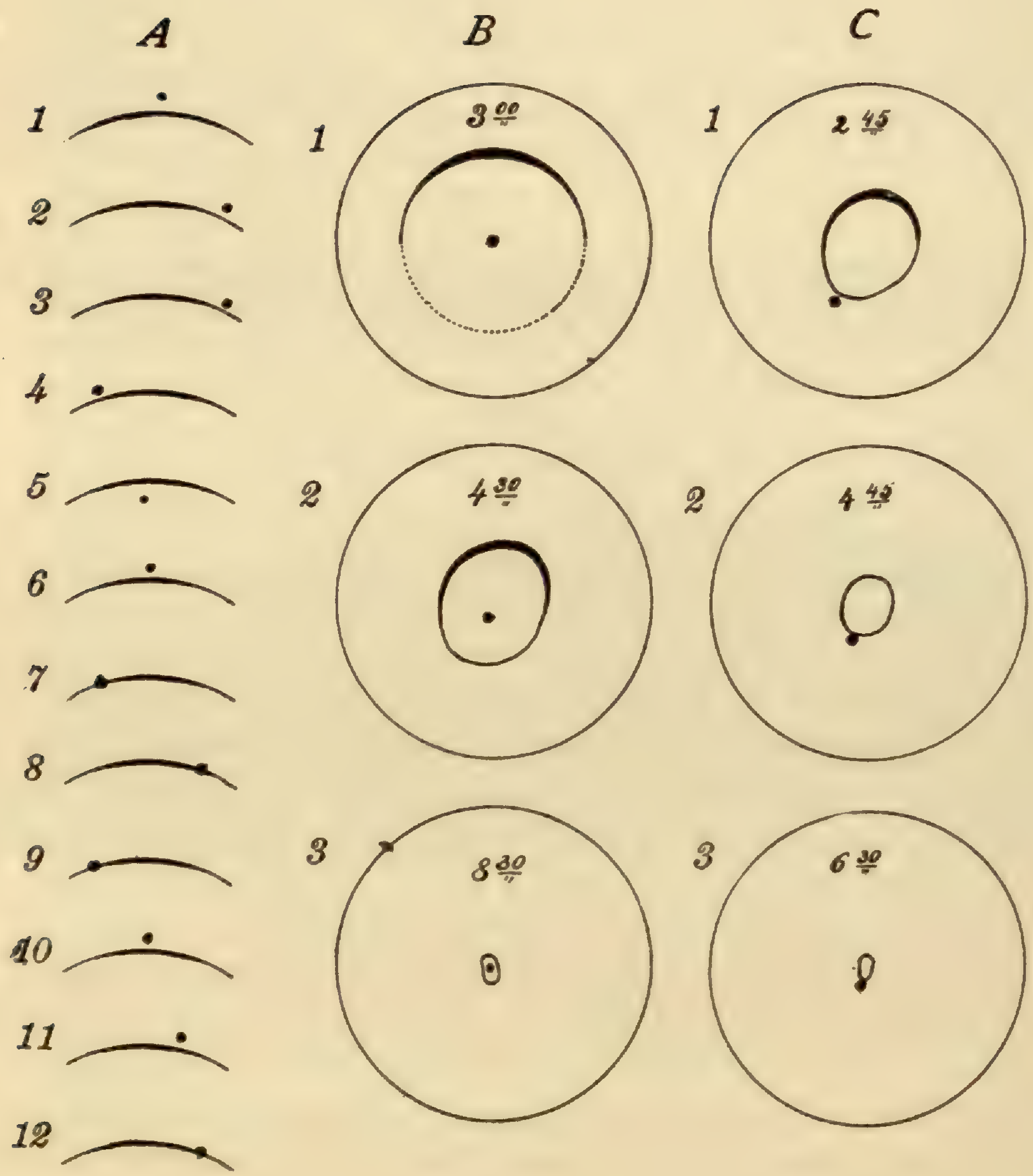
The eggs were obtained in the two-cell and four-cell stages. At this time the pigment is excentric, falling a little short of the equator on the one side and a little beyond it on the opposite. [The notes nowhere state that the antero-posterior direction of the embryo is indicated by the distribution of pigment, yet I think an examination of the figures can not fail to convince all that their interpretation can not be otherwise.—A. C. E.] When the first cleavage groove runs in the plane of symmetry the second cleavage grooves are at right angles and appear at about the same time in both halves as shown in Figs. 3 and 4. When the first cleavage groove is transverse to the plane of symmetry the second cleavage grooves do not appear at the same time, but the one on the lighter side of the upper hemisphere appears first, as shown in Fig. 1. The third cleavage usually cuts off all the pigment into the upper cells on the blastoporic (posterior) side, but leaves considerable below the upper cells on the opposite (anterior) side. The second equatorial usually cuts off all the pigmented cells on the anterior side of the egg and non-pigmented cells on the posterior (blastoporic) side.

The blastomeres on the posterior (blastoporic) side are smaller than on the anterior side, from the very first. It is the blastoporic side that takes the lead in division and the cells are smaller here all the way up to the time when the blastopore appears.

It is thus obvious that the findings by Professor Whitman not only lend confirmation to my observations on bilaterality, but that they in reality anticipate them.

It may be said with added confidence that bilaterality in the vertebrate egg is revealed through the early cleavage grooves. The cephalic portion of the embryo is indicated by the area in which cleavage grooves first appear and in which cellular division is most rapid. The caudal portion is indicated by a secondary area of cellular activity in the blastoporic region. These two areas pass into each other constituting an embryonic tract.

In addition to the above observations, Professor Whitman's manuscript and drawings give the results of a series of puncture experiments in the blastoporic lip. Since these observations have an important bearing on the question of epiboly, emboly and concrescence, they are appended.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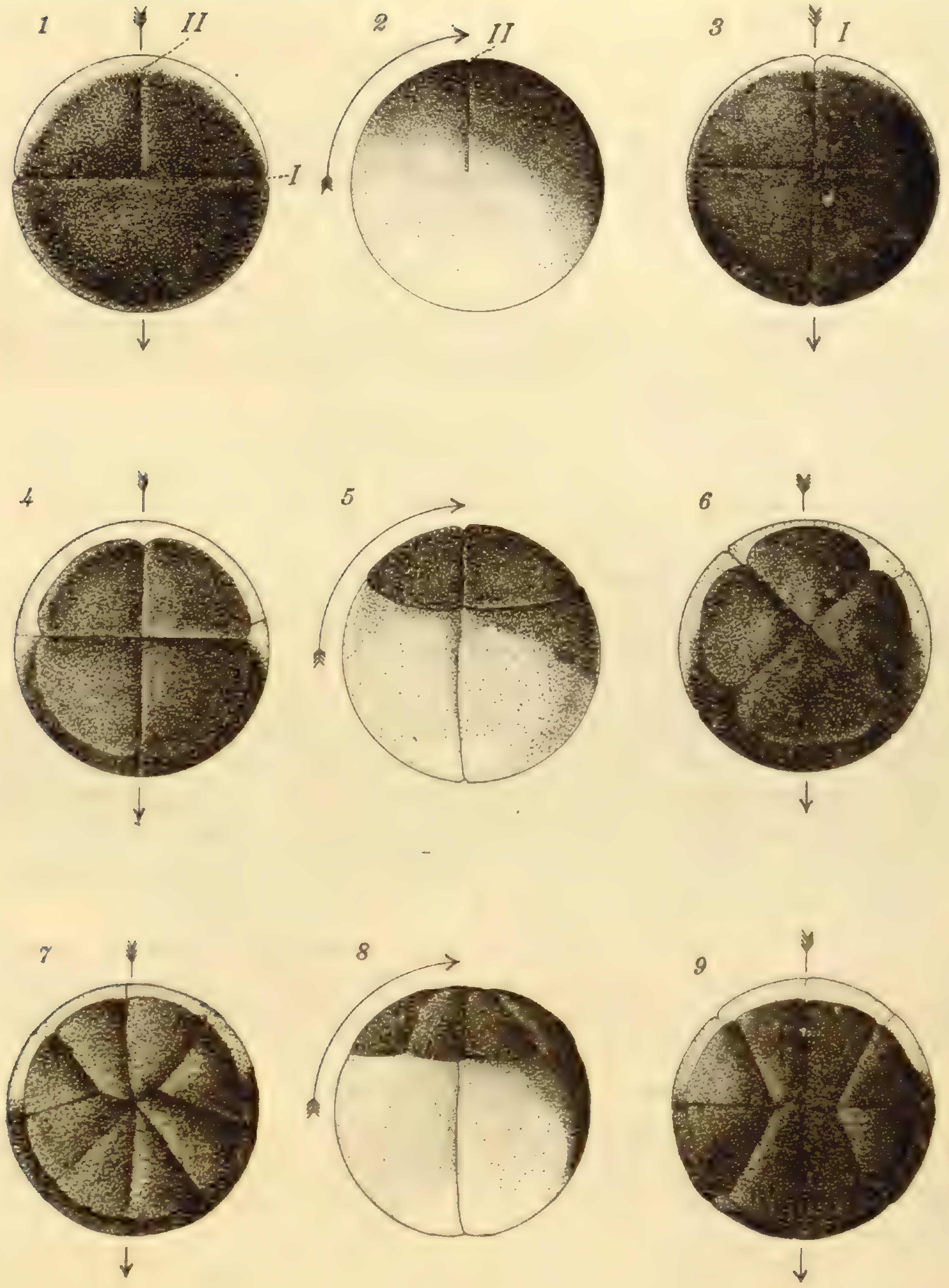
Experiments

On June 5, 1894, sixteen eggs in the thirty-two cell stage were punctured at the equator, in the middle of the white cells, as shown in Fig. 11. In twelve the blastopore appeared near the puncture as shown in the accompanying cut. The extraovates were found in the positions shown in *A*, 1–12, at 10:00 A.M. the next morning. The variations in positions are doubtless due to my punctures falling at different points, sometimes hitting as in Fig. 12, at other times in the very edge of the pigment. In the four remaining eggs two showed no extraovate and two showed no blastopore.

On June 4, 1894, pricked egg *B* at middle of lower pole, soon after the blastopore was sharply marked on the side of the embryo. Ventrally this outline was not clearly marked. At 4:30 this blastopore was outlined all around and nearly circular or about ½ diameter observed at 3:00. At 6:30 the blastopore was far advanced and nearly circular. At 8:30 it was nearly closed. It will be noted that the extraovate remained central throughout.

Another egg *C* was punctured in the ventral edge of the blastoporic rim, and the extraovate was carried along by the closing blastopore. I ought to have made two punctures, one in the middle as well, so that this approach could have been seen. However, my notes show that the blastopore advanced evenly. In this case the extraovate is carried along by this overgrowth, and one might imagine this puncture a fixed point, approached by the blastopore from the opposite side.

June 5. I pricked a number of eggs in the early cleavage stages (8–64 cells) at lower pole. In most of these eggs the extraovates were found after two to three hours to lie at or near the equator of the egg. This was long before the appearance of the blastopore. The extraovate has evidently moved and if one should leave the egg until the blastopore appeared and then look at it, it might be found at the middle of the body; and thus it might appear as if the embryo had lengthened across the lower pole (Roux). Sometimes extraovates have moved and the punctures healed.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Explanation of Plates

Since no explanation of the figures could be found other than those included in the preceding pages, I have endeavored to give an explanation in accord with the text. It should be remembered however that the figures may be open to other interpretations than those presented. The figures show the distribution of pigment and the relation of the embryo and the cleavage planes to the pigment. It will be noted that the eggs when viewed from above show a lighter area or crescent on one side. This excentric position of the pigment is likewise well shown in profile. The arrows in all cases show the direction of the forthcoming embryo.

Fig. 1. Shows the upper hemisphere of an egg in which the embryonic axis is indicated by a line passing through the centers of the light crescent and the more deeply pigmented area. In this case the first cleavage plane (I) passed at right angles to the embryonic axis. It is of interest to note that the second cleavage (II) has appeared in that portion of the egg nearest the grey crescent and further that it coincides with the median plane of the embryo.

Fig. 2. Shows a profile view of an egg in which the first cleavage coincides with the median plane of the embryo while the second is at right angles to the same. I am at a loss to understand the extent of the arrow in this and the succeeding profile views. It may be that Professor Whitman intended thus to indicate the limits of the embryonic anlage.

Fig. 3. Shows the upper hemisphere of an egg in the four cell stage. In this case the median plane of the forthcoming embryo coincides with the first cleavage groove.

Fig. 4. Shows the upper hemisphere of an egg consisting of eight cells. It is to be noted that the formation of the first equatorial sharply separates the lighter and darker portions of the egg on the one side but not on the opposite side. In this case the median plane of the embryo coincides with either the first or second cleavage groove.

Fig. 5. Represents the profile view of either the same egg or another egg in the same stage. In this case the differences in the distribution of the pigment are again shown.

Fig. 6. Shows the upper hemisphere of an egg in which neither the first nor the second cleavage grooves coincide with the median plane of the embryo.

Fig. 7. Shows the upper hemisphere of an egg at a time when the fourth cleavage grooves are present. It is impossible to say whether the median plane of the embryo coincides with either the first or the second cleavages. The appearance of the cleavage grooves leads me to infer that the direction of the arrow is parallel with either the first or the second.

Fig. 8. Represents a profile view of either the same egg or another egg in the same stage.

Fig. 9. Shows the upper hemisphere of another egg in which the fourth cleavage grooves are present. In this egg the median plane of the embryo coincides with the first or second cleavage groove.

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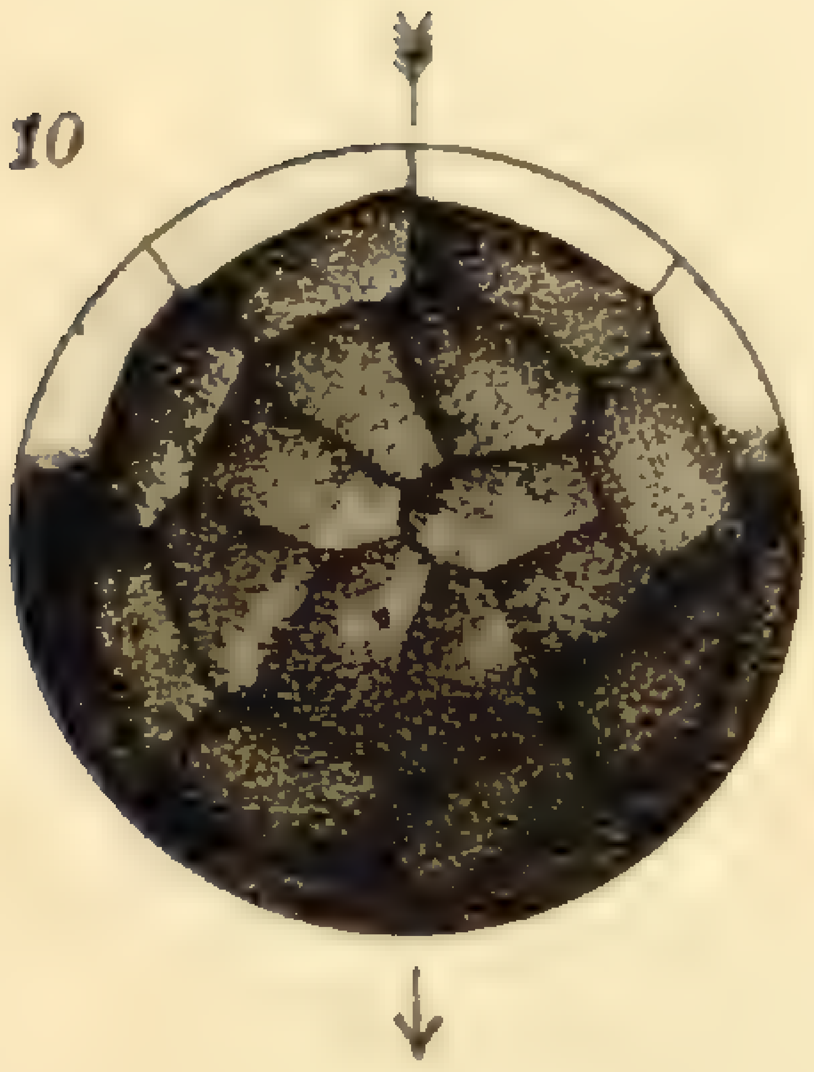

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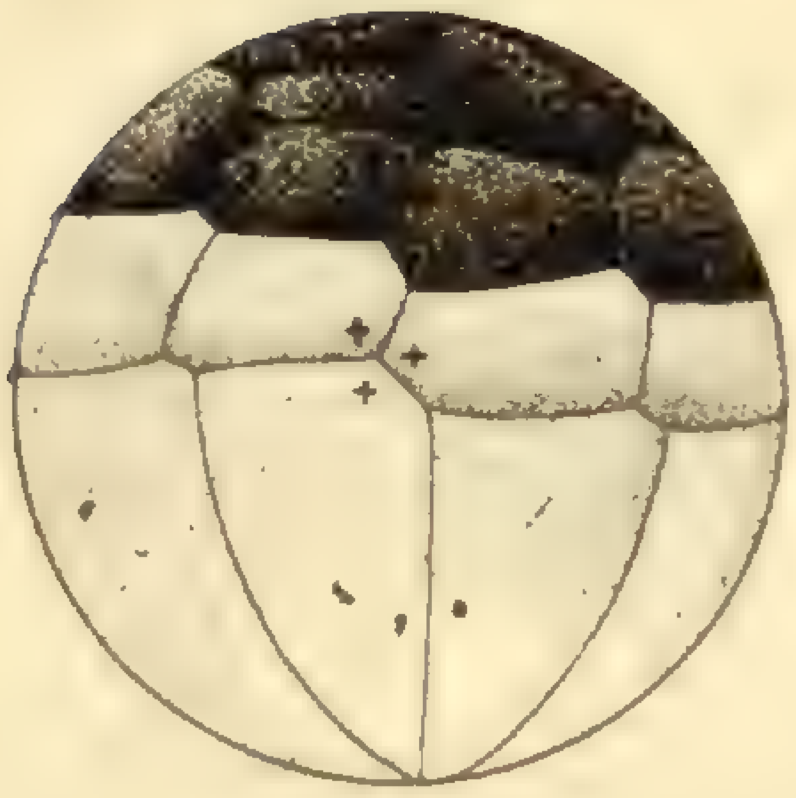

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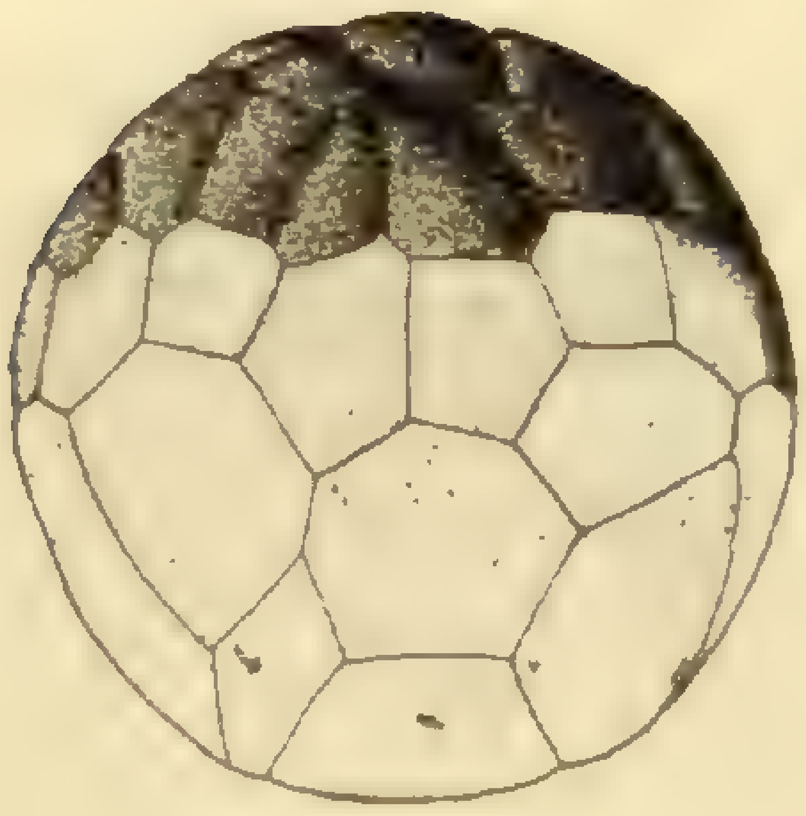

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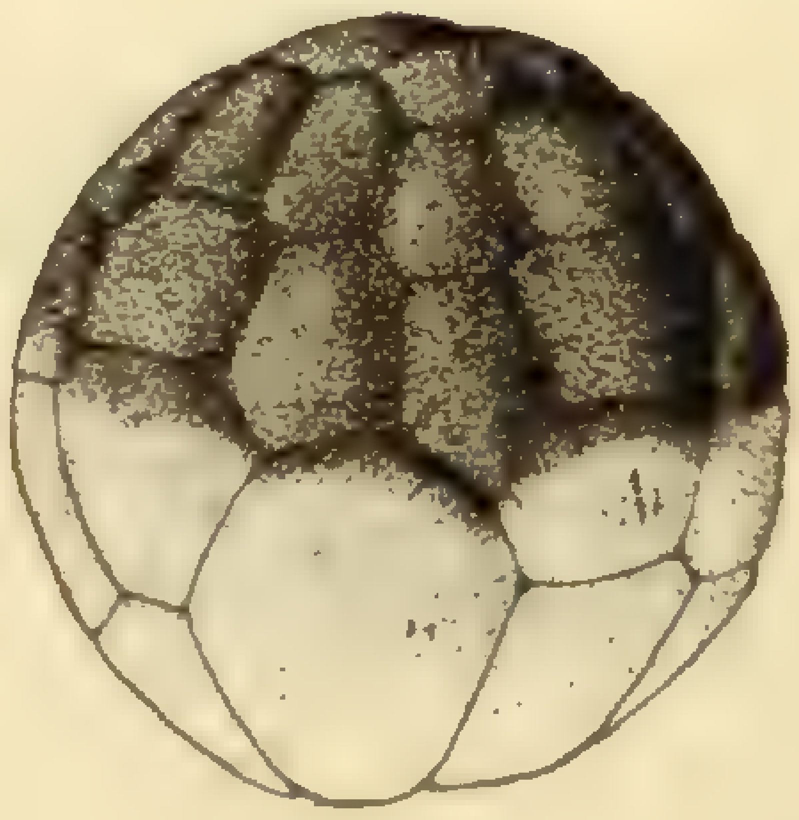

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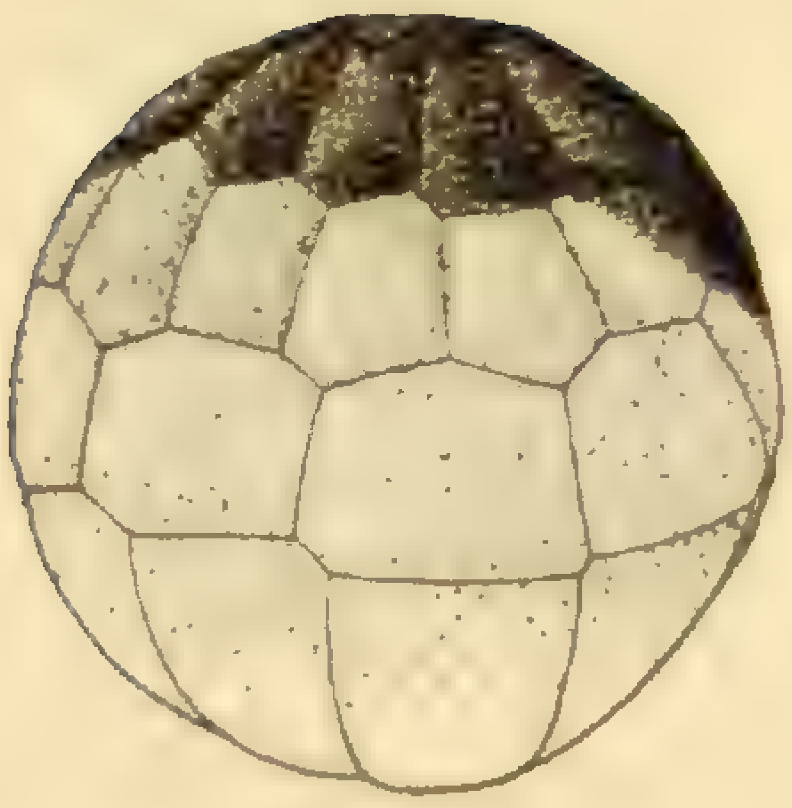

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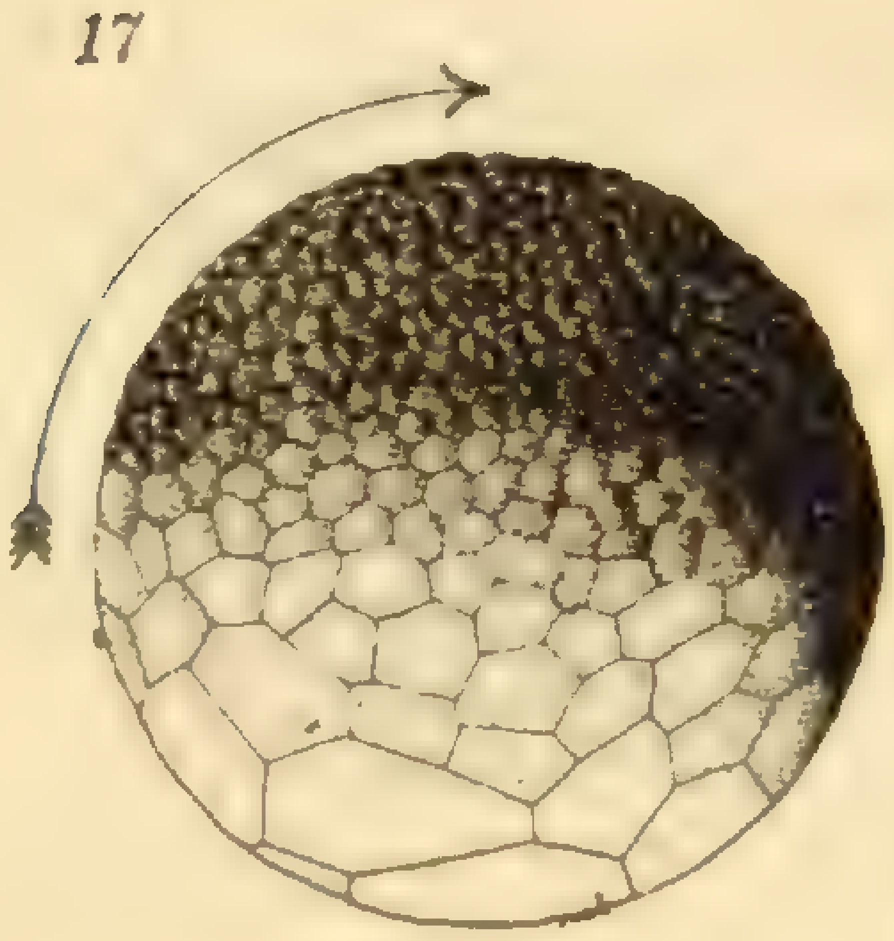

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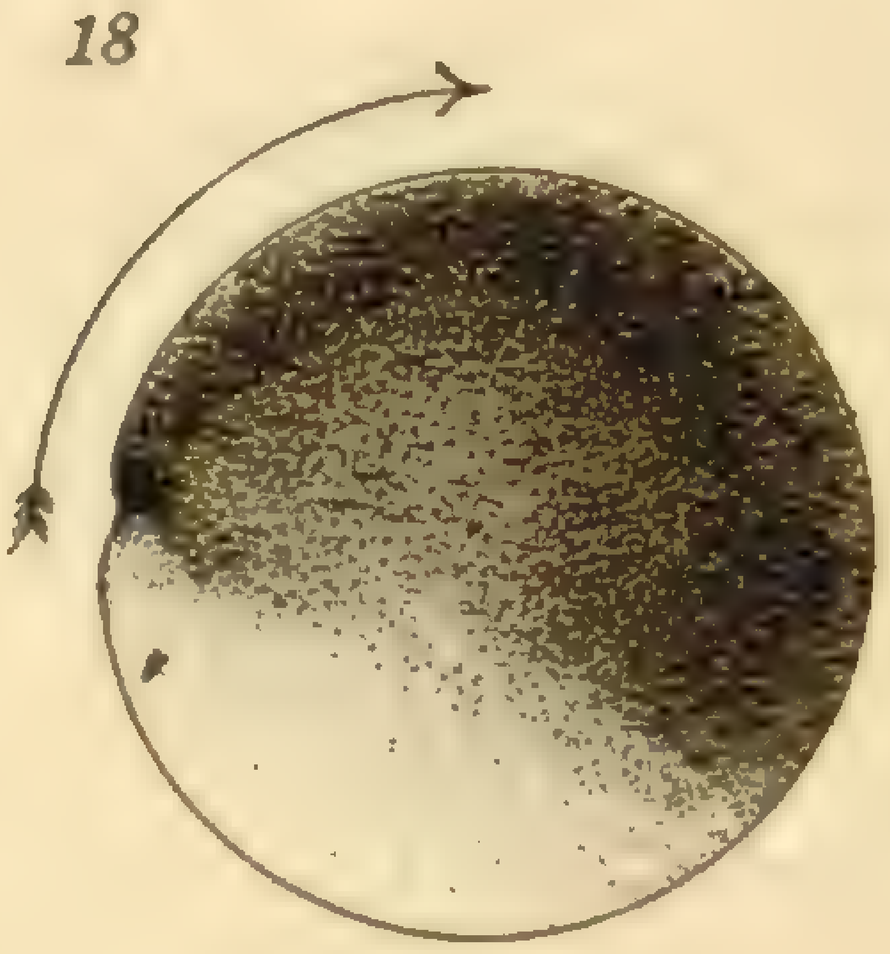

FIG. 10. Shows the upper hemisphere of an egg in a later stage of cleavage. It should be noted that the lines representing the primary grooves are entirely obscured by a shifting of the blastomeres.

FIG. 11. Represents a profile view of the same (?) egg, viewed from the side on which the blastopore is forthcoming. The small crossed lines represent the localities in which Professor Whitman punctured the eggs of this stage.

FIG. 12. Represents a profile of the opposite side of the same (?) egg.

FIG. 13. Shows the upper hemisphere of an egg in a later stage of cleavage. It should again be emphasized that it would be impossible to trace any one of the primary cleavage grooves. The cells have undergone shiftings to such a degree that if the median plane of the embryo coincided with either of the first two grooves it must be extremely irregular.

FIG. 14. Represents a profile view of the same (?) egg viewed from the side in which the blastopore will later appear. On this side cell division is decidedly in advance of the opposite side.

FIG. 15. Represents a profile view of the opposite side of the same (?) egg.

FIG. 16. ?

FIG. 17. Represents a profile view of an egg in late segmentation. The side in which the blastopore will appear is now indicated not only by the distribution of pigment but also by a decided acceleration in cell division.

FIG. 18. Represents a profile view of an egg at the time when the blastopore appears. The figure shows that it appears on the side of the egg which is least pigmented.

SHORTER ARTICLES AND DISCUSSIONS

THE TORTOISESHELL CAT

In *The Journal of Genetics* (June, 1913), Doncaster has summarized genetic data dealing with the tortoiseshell cat. The records are collected from fancy breeders and from the work of Dr. C. C. Little.

Aside from certain disputed points the inheritance is in accordance with simple sex-linkage and is analogous to the human defects—color-blindness, night-blindness, nystagmus, and hemophilia, and to the thirty or more sex-linked factors of *Drosophila*.

If the factor for yellow be represented by Y and its allelomorph, the factor for black, by B, the lack of either by b, the sex factor by X, and the allelomorph of X by x, the normal zygotic possibilities are as follows: YX — bx = yellow male. BX — bx = black male. YX — YX = yellow female. BX — BX = black female. YX — BX = tortoiseshell female.

It is obvious then that there can be but two classes of males, while there are three classes of females. Difficulties arise when it is attempted to explain the occurrence of black females produced either by the mating of a black female to a yellow male which should give only tortoiseshell females and black males, or by the mating of a tortoiseshell female to a yellow male, which should give only tortoiseshell and yellow females and black and yellow males. The occurrence of the rare tortoiseshell male is also the cause of considerable difficulty. In one mating out of seventeen of yellow females to yellow males there were produced three tortoiseshell females. There are recorded in addition from the seventeen matings forty yellow females and forty-eight yellow males which are in agreement with expectation.

In order to explain these discrepancies it is suggested that possibly the linkage of Y with X is not absolute. Yellow males may then produce gametes bX and Yx in addition to the normal or more frequent gametes YX and bx. Gamete bX is female determining, while gamete Yx is male determining and yellow bearing. The latter gamete should produce a tortoiseshell male when it meets an egg BX.

On this hypothesis we should expect the tortoiseshell males to be as frequent as the anomalous black females from yellow fathers. From the matings recorded there are eighteen anomalous black females and only three tortoiseshell males, and one of these tortoiseshell males had a black father. There is a fur-

ther objection to this hypothesis inasmuch as it is not explained how gamete bX differs from BX. Doncaster admits these difficulties, stating that further work is necessary before a definite conclusion can be reached.

In a more recent paper[1] Doncaster has suggested non-disjunction of the sex-chromosomes in oogenesis as a possible explanation. This explains the matroclinous black females, but fails to account for the lack of an equal number of patroclinous yellow males. It also fails to account for the tortoiseshell male and the occurrence of tortoiseshell females among the offspring of yellow by yellow.

In a series of experiments begun upon cats at the University of Pennsylvania during the last year, the tortoiseshell problem has been especially investigated. A yellow Persian male was crossed with common cats—black, maltese and tabby. The results, although not at present extensive, are sufficient to explain, at least in part, the anomalies observed, and to suggest a simple explanation for the occurrence of unexpected classes.

When the yellow male was crossed with a maltese female, a maltese male and two blue and cream females were produced. The blue and cream is the maltese or dilute tortoiseshell. When mated to a black female the yellow male produced both dark and dilute kittens. This shows that the black female was heterozygous for dilution. Two of the males were black and two maltese. The two females were dark tortoiseshell. When the yellow male was crossed with a dark tabby, there were produced dark and light tabbies and maltese. Blacks are also to be expected from this mating. The mother is evidently hybrid between tabby and black and between black and maltese. The female offspring showed yellow: the male offspring were without yellow except for tabby striping.

The female offspring obtained from these matings may be arranged in a series, ranging from one that is predominantly yellow to one that is maltese except for a few cream-colored hairs. The maltese with a few cream hairs occurred in the litter of three above mentioned, which included also a maltese male and a maltese female with a small cream patch.

It may be readily understood how a maltese cat with a few cream hairs or its intense form, a black cat with a few yellow hairs, would be recorded as maltese or black, and it is reasonable to suppose that further segregation of distribution factors in the direction of black would have produced a fully black female. This may

[1] *Quarterly Journal of Microscopical Science*, February, 1914.

be compared with conditions in the guinea-pig in which yellow spotting is continuous with total black. The essential differences are that in the cat we have a factor for yellow allelomorphic to a factor for black, that these allelomorphs are sex-linked, and that either alone is sufficient to produce its expected color, but that when one is balanced against the other, as in the tortoiseshell female, other factors governing the relative amounts of the two colors can act and produce continuous variation from yellow to black.

The three tortoiseshell females from the mating of yellow by yellow may be explained by supposing that the mother was gametically a tortoiseshell plus a sum of yellow extension factors and minus a sum of black extension factors.

The occurrence of the tabby factor brings in a restriction of the black pigmentation producing yellow stripes. It is therefore much more difficult to distinguish a tabby from a tabby-tortoiseshell than a black from a tortoiseshell. We have had a few tabby-tortoiseshells that would have been recorded as tabbies if close examination had not been made.

Another source of error in records involving the tortoiseshell pattern may be introduced by the occurrence of white spots. Doncaster makes no mention of these in his paper, so that it is possible that they did not occur in the animals recorded. In what is genetically a tortoiseshell and white cat the incidence of the white spotting may happen to be at just those points which would otherwise be yellow. Thus the occurrence of black and white daughters from yellow males may be explained. It is possible also that the yellow mother of the three tortoiseshell kittens recorded from the mating of yellow by yellow may have been white at points which, if pigmented, would have been black. She would then have been genetically a tortoiseshell and white and some tortoiseshell kittens would have been expected.

I would suggest as a plausible hypothesis that the rare tortoiseshell male is genetically a yellow with an extreme of black extension factors or a black with an extreme of yellow extension factors. This hypothesis is rendered more probable by some slight evidence showing that male tortoiseshells breed like yellows.

There is then no need for assuming in the cat either breaks in sex-linkage or non-disjunction of the sex chromosomes in oogenesis.

PHINEAS W. WHITING
UNIVERSITY OF PENNSYLVANIA

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A STUDY OF ASYMMETRY, AS DEVELOPED IN THE GENERA AND FAMILIES OF RECENT CRINOIDS

AUSTIN H. CLARK

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Preface

In the animal kingdom there are few, if any, forms which can be properly described as perfectly symmetrical, either from a bilateral or a radial standard. We have, however, become accustomed to refer to many types as "asymmetrical." In the sense in which we employ this word we do not intend to convey the meaning that these types alone of their respective classes depart from true bilateral or radial symmetry, but rather to indicate that they exhibit more asymmetry than the maximum contemplated in our generalized concept of, or arbitrary standard for, those classes.

Thus we readily recognize and confess the asymmetry in the skull of the narwhal (*Monodon*) with its single greatly elongated and twisted incisor, and the asymmetry in the bones in the skull of the whales, while at the same time we commonly consider man to be symmetrical, though careful measurement shows the right arm and

hand to be larger than the left, and the left leg and foot to be larger than the right.

It is clear, therefore, that in dealing with asymmetry in any group we must work inward from the most asymmetrical types toward the least asymmetrical, arbitrarily erecting a barrier between what we call asymmetry and what we are pleased to consider as "symmetry" at any point we choose.

Asymmetry—that is to say the maximum departure from perfect bilateral or radial symmetry—appears to follow certain definite lines wherever it appears, quite regardless of the type of animal, or the form, in which it is manifested.

In the following pages we shall consider the wider variations from the typical pentamerous symmetry among the recent crinoids, which is phylogenetically most extensively developed at the consummation of the phylogenetic lines, and physico-economically most extensively developed in the situations most unsuited to crinoidal existence, particularly in the very warm water of the East Indian and north Australian littoral, and the very cold water of the Antarctic regions and the deep abysses of the oceans, and is least evident among phylogenetically conservative types, and in the situations which appear to be best suited for crinoid life.

As an indication of the possible fundamental importance of the light thrown on the study of asymmetry by an examination of the data offered by the recent crinoids, it may be noticed and borne in mind that among the mammals the phylogenetically aberrant asymmetrical narwhal (*Monodon*) is exclusively arctic; the phylogenetically aberrant asymmetrical whales occupy a habitat very aberrant for the class; and the anthropoid apes, which are pronouncedly right or left handed, live in very warm regions; that among the birds the curious crook-billed plover (*Anarhynchus*), with the beak twisted to the right and one side of the body lighter in color than the other, occurs only in New Zealand, the home of many phyloge-

netic oddities; the hornbill *Rhinoplax,* with an asymmetrical tail, further peculiar in having a solid casque, an elongate central rectrix, and a naked patch on the back extending to the sides of the head, is found in the warm Malayan region; the crossbills (*Loxia*), with the tips of the mandibles crossed and a corresponding distortion in the bones of the head are all subarctic or cold temperate forms; and the owls with one ear greatly larger than the other, so far as has been determined are, like the crossbills, birds of the colder regions; and that among the fishes and similar types the very asymmetrical *Anableps* lives in the warm tropical littoral, while the flatfishes (Pleuronectidæ) are chiefly developed in the warm tropical littoral, and in cold and shallow water, and the asymmetrical forms of "Amphioxus" (using the term in its broadest sense) occur in warm and shallow water.

Further it is interesting to recall that animals under domestication—that is, living under conditions which typically lead to a more or less degenerate diversity in form and color—commonly develop asymmetry of action which, though usually occurring in the form of individual variation, may become very marked as in the case of the Japanese waltzing mice, as well as pronounced, though irregular and sporadic, asymmetry in color pattern, dentition, and other features.

More or less pronounced asymmetry undoubtedly exists in many types in which up to now it has been overlooked, and the conclusions reached in the present paper may be modified somewhat when a better knowledge of the subject is attained; but on the other hand it is scarcely probable that many instances of marked asymmetry have escaped the notice of naturalists.

The Different Types of Crinoidal Asymmetry

In the great majority of the recent crinoids the body is almost perfectly pentamerous, being composed of five similar sectors. The presence of a small muscular cone in the posterior interradius, at the summit of which is

the posterior opening of the spiral digestive tube, gives the only visible indication of a departure from true pentamerous symmetry.

In certain types, however, a more or less marked deviation from the characteristic symmetry occurs. This deviation follows four different lines:

1. A rearrangement of the five primary groove trunks upon the disk whereby (*a*) the left posterior increases in size and gives off more branches than any of the others; (*b*) as a result of the anterior migration of the mouth, the two posterior become much longer and the anterior much shorter than the others and a condition of bilateral symmetry is attained; (*c*) correlated with the anterior migration of the mouth, all of the primary groove trunks become merged into a horse-shoe shaped ring which skirts the lateral and anterior borders of the disk, giving off branches to the arms, the mouth being in the right center of the ring so that the ambulacra on the left are more developed than those on the right, or the ambulacra leading to the left posterior arm disappearing altogether so that the ambulacra on the right are more developed than those on the left;

2. A dwarfing, or an overdevelopment, of the left posterior, more rarely of both, posterior radials with their post-radial series;

3. The intercalation of additional radials and post-radial series which alternate with the original five, and the associated dropping out of one of the five radials; and

4. The suppression of two of the primarily five basals.

The Asymmetrical Crinoids

In the following list are given all the families and genera of recent crinoids which include asymmetrical species.

After the families the bathymetrical and thermal ranges are given, and after the genera the bathymetrical range.

Certain families are represented in the warm littoral

water of the Malayan region and northern Australia, but the highest actual temperature record is considerably less than the temperature of this water; in these cases the temperature 80.5° is given after the ascertained maximum as more nearly representing the true maximum.

Of the nine families the four in which asymmetry is most markedly developed are marked with an asterisk (*); and of the twenty-seven genera the sixteen which include the most notably asymmetrical species are similarly distinguished.

	Depth (Fathoms)	Temperature (F.)
Capillasterinæ	0–830	44.5–78.5 (80.5)
Comatella	0–106	
Neocomatella	10–830	
Palæocomatella	140–153	
Capillaster	0–160	
Nemaster	0–194	
Comissia	0–100	
Leptonemaster	42–163	
Comactiniinæ	0–288	62.0–71.9 (80.5)
**Comatula*	0–160	
Comatulella	0–10	
Cominia	0–288	
Comactinia	0–262	
*Comasterinæ	0–140	52.3–80.0 (80.5)
**Comaster*	0–95	
**Comantheria*	0–83	
**Comanthina*	0–42	
**Comanthus*	0–140	
Heliometrinæ	2–1,600	28.7–60.5
**Promachocrinus*	10–222	28.7
Pentametrocrinidæ	103–1,800	33.5–60.6
*Thaumatocrinus	361–1,800	
*Apiocrinidæ	565–940	36.7–38.1
**Proisocrinus*	940	
**Carpenterocrinus*	565	
Bourgueticrinidæ	62–2,690	29.1–70.7
**Rhizocrinus*	77–1,300	32.2–48.7
Monachocrinus	687–2,419	37.4–40.0
*Holopodidæ	5–120	71.0
**Holopus*	5–120	
*Plicatocrinidæ	266–2,575	31.1–43.9
**Calamocrinus*	392–782	
**Ptilocrinus*	266–2,485	
**Hyocrinus*	1,600–2,575	
**Gephyrocrinus*	992–1,103	
**Thalassocrinus*	1,262–2,325	

The Phylogenetic Distribution of Asymmetry

The phylogenetic distribution of the asymmetry among the recent crinoids is very interesting.

Asymmetry is almost universal in the comatulid family Comasteridæ, which includes the most specialized of all recent forms; in this family the first and second types occur, though the latter is much less common.

Asymmetry is characteristic of the genus *Promachocrinus,* which is probably rightly considered as the most specialized genus in the subfamily Heliometrinæ, the largest and most universally distributed subfamily of the at present dominant family Antedonidæ; in the genus *Promachocrinus* the first and third types occur.

Asymmetry is equally characteristic of the genus *Thaumatocrinus,* the most specialized genus of the family Pentametrocrinidæ; in this genus the third type is found.

Asymmetry exists in all of the genera of the Plicatocrinidæ, which includes the last highly specialized exponents of the ancient order Inadunata, which flourished from the Ordovician to the Carboniferous, with one family extending into the Permian and Trias and another (the present family) appearing in the Jura; in the Plicatocrinidæ the first, second and fourth types occur in recent genera, while the third is also found in fossil genera.

Asymmetry is characteristic of both of the recent genera of Apiocrinidæ, which are the most specialized genera in the family; in these the second type occurs.

Asymmetry of the second type is characteristic of the only recent genus of the Holopodidæ.

Asymmetry characterizes both of the species of *Rhizocrinus*—which is at least as highly specialized as any of the genera of the Bourgueticrinidæ—existing in the present seas, and one of the species of *Monachocrinus,* a genus of which the exact phylogenetic position is uncertain, although it is probably on a par with *Rhizocrinus;* in these the third type occurs.

In the following list the recent asymmetrical types are

given in the order of the extent of their departure from the normal pentamerous symmetry:

Plicatocrinidæ:	Asymmetry of Types 1, 2, (3) and 4.
Comasteridæ:	Asymmetry of Types 1 and 2.
Promachocrinus:	Asymmetry of Types 1 and 3.
Apiocrinidæ:	Asymmetry of Type 2.
Holopodidæ:	Asymmetry of Type 2.
Thaumatocrinus:	Asymmetry of Type 3.
Rhizocrinus:	Asymmetry of Type 3.
Monachocrinus:	Asymmetry of Type 3.

The asymmetry of the Comasteridæ is considered more fundamental than that of *Promachocrinus* for the reason that it is characteristic of practically the entire family, and also because it results in a much greater degree of irregularity. It is interesting to note that asymmetry of Type 3 is not uncommon among the Comasteridæ, in the form of individual variation.

The asymmetry of the Apiocrinidæ and Holopodidæ is considered more fundamental than that of the genus *Thaumatocrinus* for the reason that it affects the entire family, at the same time inducing a greater departure from the normal form.

The asymmetry of *Rhizocrinus* is considered less fundamental than that of *Thaumatocrinus* because, though affecting all of the species, exactly as in *Thaumatocrinus,* it is less extensively developed.

The asymmetry of *Monachocrinus* affects only one of the seven species of the genus.

Briefly stated, it appears that, no matter in what form it may manifest itself, metameric asymmetry in the recent crinoids is an attribute of the most specialized types in the groups in which it occurs.

From the conditions in the Plicatocrinidæ, the last remnants of the once abundant Inadunata, it would appear that asymmetry is an attribute of phylogenetically decadent types—types in which type senescence has so far advanced as to inhibit the normal course of development.

The Geographical Distribution of Asymmetry

The geographical distribution of asymmetry is as interesting as the phylogenetical distribution.

Although occurring everywhere except in the Arctic Ocean and in the Mediterranean, Bering, Okhotsk and Japan seas, asymmetrical types are most frequent and most highly developed (1) in warm shallow water from southern Japan southward throughout the Malay Archipelago to northern Australia and westward to Ceylon, and (2) in the Antarctic and in the cold abysses.

Though present among species inhabiting the west Atlantic from North Carolina to Brazil, and characteristic of many forms living at intermediate depths in the western Pacific and in the Indian Oceans, in these it is never more than slightly developed, even though they be very closely related to types in which it is, in other situations, carried to an extreme.

Depth (Fathoms)	Number of Asymmetrical Genera	Number of Symmetrical Genera	Per Cent. of the Latter Represented by the Former
0–50	16	50	32%
50–100	15	53	28
100–150	13	51	25
150–200	10	44	22
200–250	5	39	13
250–300	5	34	14
300–350	3	30	10
350–400	4	32	12
400–450	5	29	17
450–500	5	27	18
500–550	5	26	19
550–600	6	26	23
600–650	5	26	19
650–700	6	22	27
700–750	6	22	27
750–800	6	18	33
800–850	5	18	28
850–900	4	18	22
900–950	5	19	26
950–1,000	5	16	31
1,000–1,100	5	16	31
1,100–1,200	5	12	41
1,200–1,300	5	9	55
1,300–1,400	4	9	44
1,400–1,500	4	7	57
1,500–1,600	4	7	57
1,600–1,700	5	3	166
1,700–1,800	5	3	166
1,800–1,900	4	3	133
1,900–2,000	4	3	133
2,000–2,500	4	3	133
2,500–3,000	1	3	33

In short, though almost universal, occurring every-

FIG. 1. The Relation between the Genera with and without Asymmetrical Species at Different Depths (———), with very Asymmetrical and without Asymmetrical Species at Different Depths (═══), and the Decrease in the Number of Genera with Depth, expressed in Percentages of the Total Number (- - - -).

where except in inland seas, asymmetry is especially developed in the warm waters of the eastern tropics, particularly in the Malayan region and in northern Australia, and in the Antarctic and the cold abysses.

Bathymetrical Distribution of the Asymmetrical Crinoids

The number of genera of recent crinoids including asymmetrical species, the number of genera including

Depth (Fathoms)	Number of Asymmetrical Genera Which Are Marked *	Number of Symmetrical Genera	Per Cent. of the Latter Represented by the Former
0–50	7	50	14.0
50–100	7	53	13.2
100–150	5	51	9.8
150–200	3	44	6.8
200–250	2	39	5.1
250–300	2	34	5.8
300–350	2	30	6.6
350–400	4	32	12.5
400–450	4	29	13.8
450–500	4	27	14.8
500–550	4	26	15.4
550–600	5	26	19.2
600–650	4	26	15.4
650–700	4	22	18.1
700–750	4	22	18.1
750–800	4	18	22.2
800–850	3	18	16.6
850–900	3	18	16.6
900–950	4	19	21.0
950–1,000	4	16	25.0
1,000–1,100	4	16	25.0
1,100–1,200	4	12	33.3
1,200–1,300	4	9	44.4
1,300–1,400	3	9	33.3
1,400–1,500	3	7	42.8
1,500–1,600	3	7	42.8
1,600–1,700	4	3	133.3
1,700–1,800	4	3	133.3
1,800–1,900	3	3	100.0
1,900–2,000	4	3	100.0
2,000–2,500	3	3	100.0
2,500–3,000	1	3	33.3

only symmetrical species, and the percentage of the number of symmetrical genera represented by the number of asymmetrical genera at different depths are given in the table on page 528 and shown in Fig. 1.

Considering the percentages only, these may be regrouped as follows:

0– 200	27
200– 650	16
650–1,100	28
1,100–3,000	92

Considering only the genera marked with an asterisk (*) we find the representation at different depths given in the table on page 530 and in Fig. 1.

Considering the percentages only, these may be regrouped, as follows:

0– 100	13.6
100– 350	6.8
350– 900	16.6
900–3,000	61.8

The number of families of recent crinoids including asymmetrical species, the number of families including only symmetrical species, and the percentage of the number of families including only symmetrical species represented by the number of families including asymmetrical species at different depths, are shown in the table on page 533 and in Fig. 2.

The proportion of the genera including asymmetrical species to those composed entirely of symmetrical species, about one third between the shore line and 50 fathoms, decreases to a minimum of one tenth at from 300 to 350 fathoms, and then increases, with greater and greater rapidity, to 1,600 fathoms and below.

It is everywhere less than one quarter between 100 and 650 fathoms. Thus it is evident that the genera including asymmetrical species are chiefly developed in shallow water, and in deep water, and are least developed in water of intermediate depth.

Taking the ocean as a whole, the temperature at 100 fathoms is 60.7°, and at 650 fathoms 38.6°; the optimum temperature for the recent crinoids appears to be between 50° and 65°; when we remember that most of the asymmetrical species, and all of the most asymmetrical ones, in the genera which give us our numbers for 0–50 and for 50–100 fathoms, are confined to a littoral belt of scarcely more than 50 fathoms, it becomes at once evident that asymmetry among the crinoids is developed chiefly in

FIG. 2. The Proportion of Families with and without Asymmetrical Species at Different Depths (———), and the Percentage of the Total Number of Crinoid Families represented at Different Depths (– – – –).

Depth (Fathoms)	Number of Asymmetrical Families	The Families Marked with an *	Number of Symmetrical Families	Per Cent. of Symmetrical Families Represented by the Asymmetrical
0–50	5	2	15	33
50–100	6	2	15	40
100–150	7	2	13	54
150–200	5	0	11	45
200–250	5	0	10	50
250–300	6	0	9	66
300–350	5	0	8	62
350–400	5	0	7	71
400–450	5	0	7	71
450–500	5	0	7	71
500–550	5	0	8	62
550–600	6	1	8	75
600–650	6	1	7	86
650–700	6	1	7	86
700–750	6	1	7	86
750–800	6	1	6	100
800–850	6	1	6	100
850–900	5	1	6	83
900–950	5	1	6	83
950–1,000	4	0	5	80
1,000–1,100	4	0	5	80
1,100–1,200	4	0	5	80
1,200–1,300	4	0	4	100
1,300–1,400	4	0	4	100
1,400–1,500	4	0	3	133
1,500–1,600	4	0	3	133
1,600–1,700	3	0	1	300
1,700–1,800	3	0	1	300
1,800–1,900	2	0	1	200
1,900–2,000	2	0	1	200
1,900–2,000	2	0	1	200
2,000–2,500	2	0	1	200
2,500–3,000	2	0	1	200

water above and below the optimum, and least at and just below the optimum temperature.

Considering only the genera marked with an asterisk (*), that is, the genera with the most highly developed asymmetry, we find the same general facts emphasized as in the case of all the genera including asymmetrical forms; but here the minimum is between 50 and 400 fathoms instead of between 100 and 650 fathoms. The temperature at 400 fathoms is 41.8°. This approximation of the minimum to the zone of optimum temperature when only the most asymmetrical types are considered strengthens the hypothesis that the zone of optimum temperature really represents the zone of least-developed asymmetry.

Comparing the proportionate abundance of asymmetrical genera at different depths with the frequency of all the genera expressed as percentages of the total, we find that the former decreases while the latter increases to 50–100 fathoms; from this point the two run roughly parallel to 300–350 fathoms, after which the former increases with progressively greater rapidity, while the latter decreases steadily and gradually to 3,000 fathoms; the two cross each other between 600 and 700 fathoms.

The proportion of the families including asymmetrical species to those composed entirely of symmetrical species increases from one third at 0–50 fathoms to three times as many at 1,600 fathoms and twice as many at 1,900 fathoms and over. The increase, though irregular—largely as a result of the small numbers involved at the greater depths—is constant.

The number of families at different depths, expressed as percentages of the total number, increases from 0–50 to 50–100 fathoms, and then decreases to 1,800 fathoms and beyond. Except for a minimum between 350 and 500 fathoms the decrease is fairly regular.

The two lines cross between 200 and 300 fathoms.

The reversal of the direction of the line representing the frequency of the families including asymmetrical species as a percentage of the number of the families not including asymmetrical species at different depths, as compared with the line representing the frequency of the families at different depths expressed as percentages of the total number, indicates that the less favorable the environment for crinoids as a whole the greater becomes the proportion of asymmetrical forms.

In the proportion of genera including asymmetrical species to those composed entirely of symmetrical species we find a minimum between 100 and 650 fathoms or, considering only the most markedly asymmetrical types, between 50 and 400 fathoms, the numbers above 100 (or 50) fathoms and below 650 (or 400) fathoms being greater.

Considering families in the same light we appear to

have an increase between 350 and 500 fathoms—that is, more or less coinciding with this minimum.

In the frequency of families at different depths expressed as percentages of the total number we notice a minimum between 350 and 500 fathoms which reaches a point not again touched until 750–800 fathoms and beyond. This indicates the occurrence here of a proportionately large number of families including asymmetrical species, but at the same time a proportionately small number of genera including asymmetrical species within those families.

Thermal Distribution of the Asymmetrical Crinoids

In examining the thermal distribution of asymmetry among the recent crinoids we find it advisable to employ family instead of generic units, for the reason that our records are insufficient to furnish us with even approximate thermal ranges for many of the individual genera, though in most cases these may be estimated with reasonable exactness. The records for the crinoids of the deeper water are far more satisfactory than the records for the crinoids of the littoral, and this is very fortunate, for it justifies us in assigning a temperature of 65° and over to a number of species and genera which are of great importance in the present study.

In considering asymmetry in relation to temperature by family units it must constantly be borne in mind that, whereas certain families (Capillasterinæ, Comactiniinæ and Comasterinæ) extend from the warm littoral into moderately deep water with a relatively low temperature, the asymmetry among their component genera and species is strongly marked only in very shallow water of high temperature, and is only slightly marked—indeed not infrequently entirely absent, as in *Comatilia*—in genera and species inhabiting deep and cold water.

Thus through a study of family units the amount of asymmetry shown at intermediate temperatures is really exaggerated, and appears in its relation to the higher

and to the lower temperatures considerably greater than it really is.

In the subfamily Heliometrinæ, the largest and most widely distributed subfamily of the Antedonidæ, which itself is the dominant crinoid family of the present seas, the range of temperature is very great; but as only one out of the ten genera of the Heliometrinæ is asymmetrical it has seemed sufficient to consider and to tabulate the temperature of this genus (*Promachocrinus*) alone.

The frequency of the families including asymmetrical crinoids at different temperatures is as follows:

85°–80°	4
80–75	4
75–70	4
70–65	3
65–60	4
60–55	3
55–50	3
50–45	3
45–40	4
40–35	5
35–30	4
30–25	1

These frequencies fall into the following groups:

Below 30°	1
30–45	4.3
45–65	3.2
65–85	3.7

or,

Below 30°	1
30–45	4.3
45–70	3.2
70–85	4.0

Considering the zone of optimum temperature (50°–65°) in contrast to the temperatures above and below, and omitting the exceptionally low temperatures below 30°, we have:

30°–55°	3.8
50–65	*3.3*
65–80	4.0

Bearing in mind always that the frequency between the warm littoral and the cold abyssal temperatures is exaggerated because of the segregation in the warm littoral zone of the most asymmetrical genera and species in many of the families inhabiting intermediate temperatures, it is clear that asymmetry is least developed at the optimum temperature for crinoid life, and most developed in temperatures which are phylogenetically too warm or too cold.

This agrees perfectly with what we found from an examination of the bathymetrical distribution of asymmetry.

A comparison between the frequency of the families of crinoids represented in the recent seas, including only symmetrical species, given in the actual numbers and also as percentages of the total numbers, and the frequency of the families including asymmetrical species, given in the same way, follows (Fig. 3):

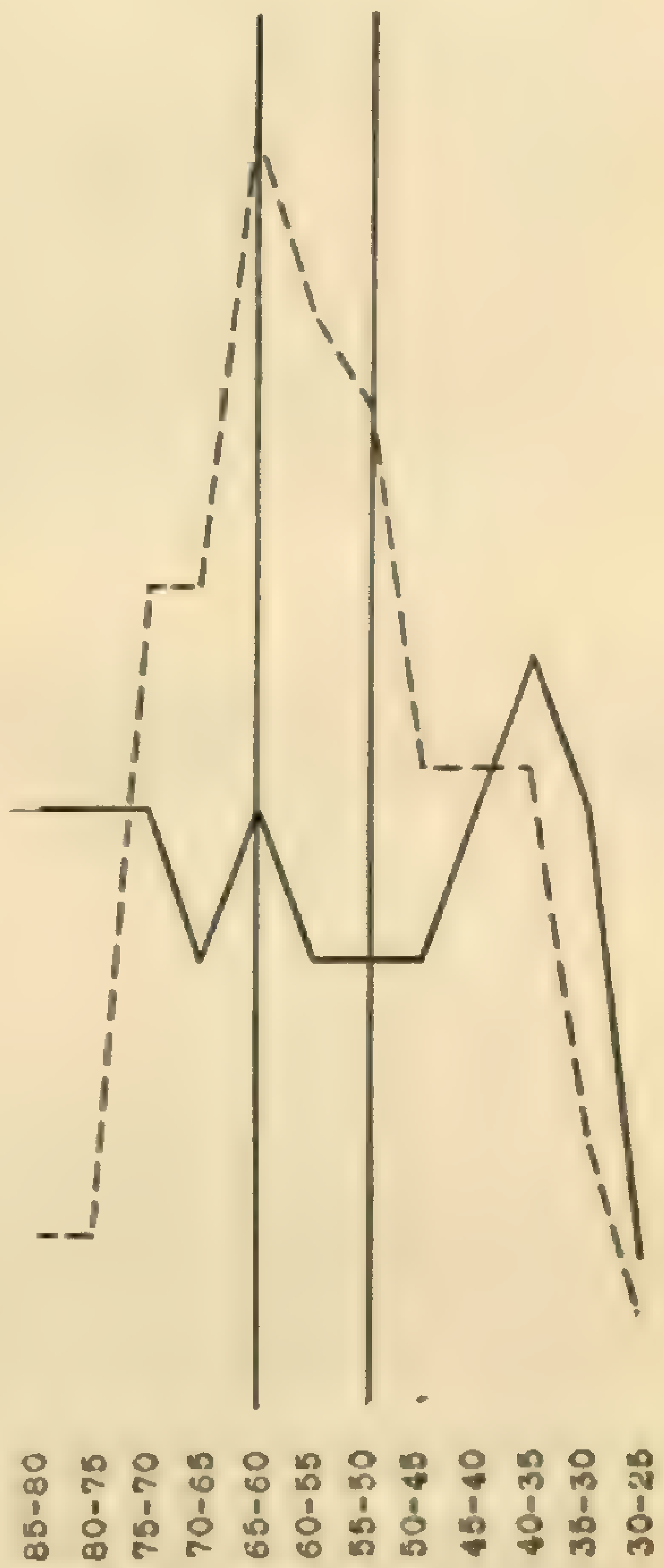

FIG. 3. Frequency at Different Temperatures of the Families including Asymmetrical Species (———), and those including Symmetrical Species only (- - - -).

Temperature (Fahrenheit)	Families with Symmetrical Species Only	Per Cent. of Total	Families with Asymmetrical Species	Per Cent. of Total
85°–80°	2	13	4	44
80 –75	2	13	4	44
75 –70	9	60	4	44
70 –65	9	60	3	33
65 –60	14	93	4	44
60 –55	12	80	3	33
55 –50	11	73	3	33
50 –45	7	47	3	33
45 –40	7	47	4	44
40 –35	7	47	5	55
35 –30	3	20	4	44
30 –25	1	7	1	11

The Asymmetrical Features in Detail

In the following list are given the four types of asymmetry occurring in the recent crinoids, with their geographical distribution and the genera in which they are found.

1. *Disk Not Radially Symmetrical*

Geographical Distribution.—Southern Japan southward to Samoa, Fiji and southern Australia, thence west-

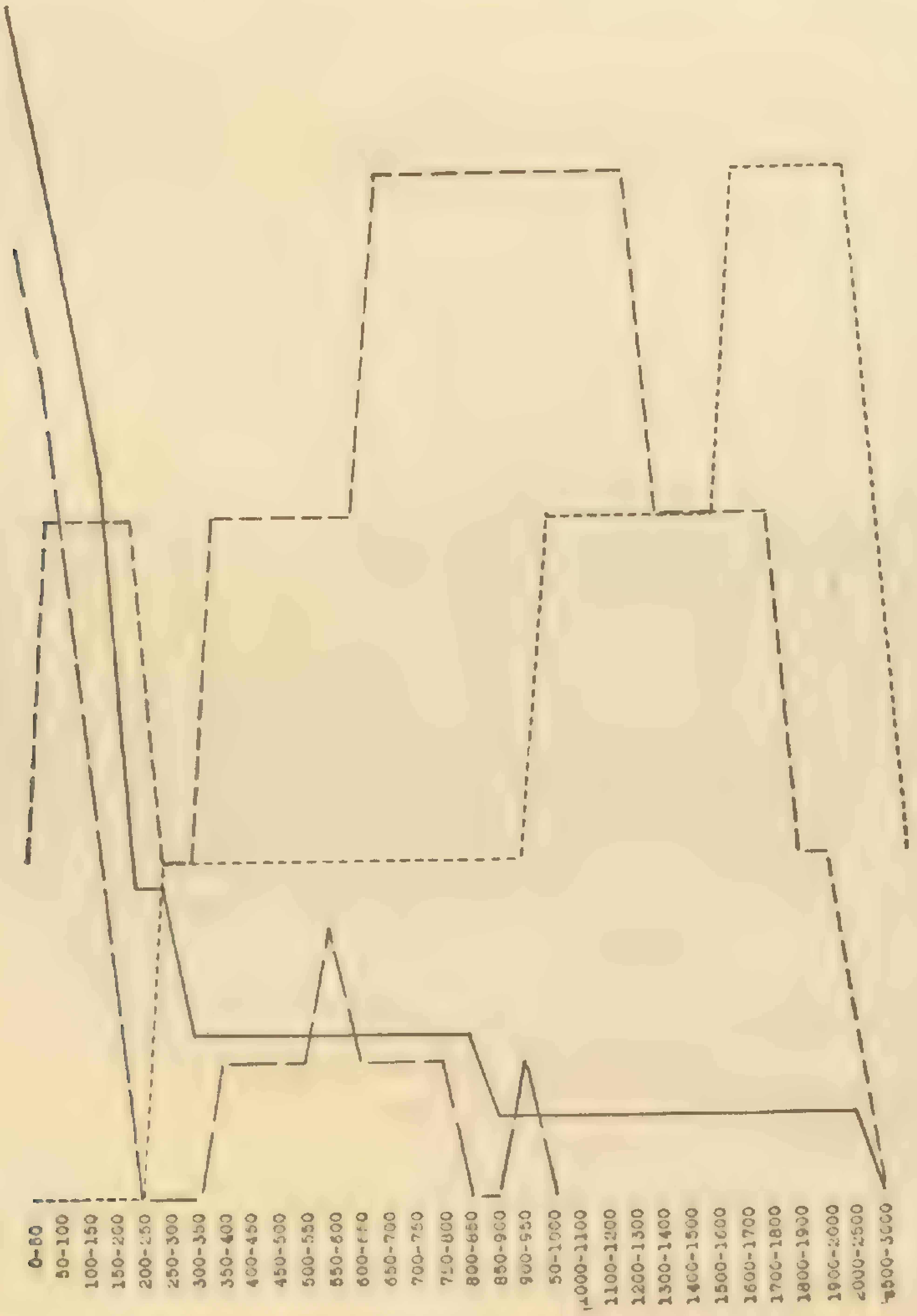

Fig. 4. Frequency at Different Depths of the Genera with Asymmetrical Disks (———), the Genera with One or More Rays Dwarfed or Enlarged (—— ——), the Genera with Six to Ten Rays (- – – –), and the Genera with Three Basals (- - - - -), expressed as Percentages of the Total Number in Each Class.

ward to east Africa, from the Red Sea to the Cape; northwestern Africa and southwestern Europe (in moderately deep water), and from South Carolina to Brazil; antarctic regions, littoral to abyssal, and northward along the

eastern shores of the Pacific (in deep water) to British Columbia.

This character is most strongly marked in the shallow water from the Marshall Islands and New Caledonia through the Malay Archipelago and along the northern coasts of Australia, and thence westward to Ceylon; and again in the antarctic regions and the abysses of the east Pacific.

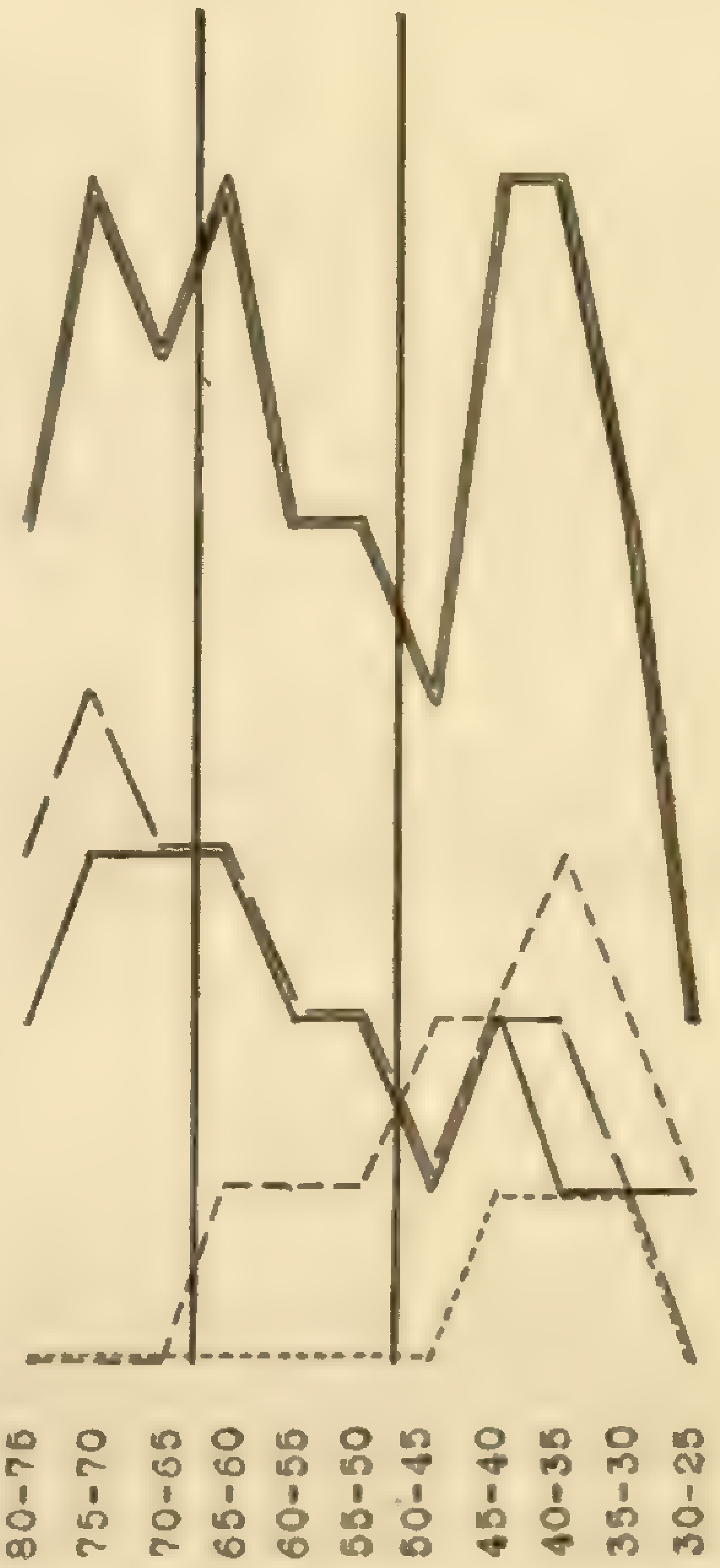

Fig. 5. Frequency at Different Temperatures of Families including Species in which the Disk is not Radially Symmetrical (———), Families including Species with one or More Rays Dwarfed or Enlarged (—— ——), Families including Species with from Six to Ten Rays (- - - -), and Families including Species with Three Basals (- - - - - -), and the Total of all these Irregularities.

Systematic Distribution.—

Capillasterinæ

Comatella *Capillaster*
Neocomatella *Nemaster*
Palæocomatella *Comissia*
Leptonemaster

Comactiniinæ

Comatula *Cominia*
Comatulella *Comactinia*

Comasterinæ

Comaster *Comanthina*
Comantheria *Comanthus*

Heliometrinæ

Promachocrinus

Plicatocrinidæ

Ptilocrinus

2. *One or More Rays Dwarfed, or Enlarged*

Geographical Distribution.—Malayan region and north Australia, and Caribbean Sea, but only in warm and shallow water; Malay Archipelago to southern Japan, and Galápagos Islands to Central America in deep cold water.

Systematic Distribution.—

Capillasterinæ

Capillaster (part)

Comactiniinæ

Comatula

Comasterinæ

Comaster (part) *Comanthina*

Comantheria (part) *Comanthus* (part)

Apiocrinidæ

Proisocrinus *Carpenterocrinus*

Holopodidæ

Holopus

Plicatocrinidæ

Calamocrinus

3. *Six to Ten (Sometimes Four) Rays*

Geographical Distribution.—Southern Japan and the Hawaiian Islands to the Malay Archipelago, in rather deep water; abysses of the Indian Ocean and the Antarctic; Florida northward and northeastward to Iceland and Norway in deep and cold water.

FIG. 6. Proportion at Different Depths of Genera only Symmetrical Species, and Genera including Species with Asymmetrical Disks (————), Genera including Species with One or More of the Rays Dwarfed or Enlarged (—— ——), Genera including Species with from Six to Ten Rays (— — -), and Genera including Species with Three Basals (------).

This feature as an individual variant occurs in the warm water of the Malayan region, in the shallower portions of the Caribbean Sea, and very commonly on the tropical Brazilian coast.

Depth (Fathoms)	Disk Not Radially Symmetrical (17)	One or More Rays Dwarfed or Enlarged (10)	Six to Ten Rays (4)	Three Basals (4)	Total (35)
0–50	(15) 88	(7) 70	(1) 25	0	(23) 66
50–100	(13) 76	(6) 60	(2) 50	0	(21) 60
100–150	(11) 65	(4) 40	(2) 50	0	(17) 49
150–200	(9) 53	(2) 20	(2) 50	0	(13) 37
200–250	(4) 23	0	(2) 50	0	(6) 17
250–300	(4) 23	0	(1) 25	(1) 25	(6) 17
300–350	(2) 12	0	(1) 25	(1) 25	(4) 11
350–400	(2) 12	(1) 10	(2) 50	(1) 25	(6) 17
400–450	(2) 12	(1) 10	(2) 50	(1) 25	(6) 17
450–500	(2) 12	(1) 10	(2) 50	(1) 25	(6) 17
500–550	(2) 12	(1) 10	(2) 50	(1) 25	(6) 17
550–600	(2) 12	(2) 20	(2) 50	(1) 25	(7) 20
600–650	(2) 12	(1) 10	(2) 50	(1) 25	(6) 17
650–700	(2) 12	(1) 10	(3) 75	(1) 25	(7) 20
700–750	(2) 12	(1) 10	(3) 75	(1) 25	(7) 20
750–800	(2) 12	(1) 10	(3) 75	(1) 25	(7) 20
800–850	(2) 12	0	(3) 75	(1) 25	(6) 17
850–900	(1) 6	0	(3) 75	(1) 25	(5) 14
900–950	(1) 6	(1) 10	(3) 75	(1) 25	(6) 17
950–1,000	(1) 6	0	(3) 75	(2) 50	(6) 17
1,000–1,100	(1) 6	0	(3) 75	(2) 50	(6) 17
1,100–1,200	(1) 6	0	(3) 75	(2) 50	(6) 17
1,200–1,300	(1) 6	0	(3) 75	(2) 50	(6) 17
1,300–1,400	(1) 6	0	(2) 50	(2) 50	(5) 14
1,400–1,500	(1) 6	0	(2) 50	(2) 50	(5) 14
1,500–1,600	(1) 6	0	(2) 50	(2) 50	(5) 14
1,600–1,700	(1) 6	0	(2) 50	(3) 75	(6) 17
1,700–1,800	(1) 6	0	(2) 50	(3) 75	(6) 17
1,800–1,900	(1) 6	0	(1) 25	(3) 75	(5) 14
1,900–2,000	(1) 6	0	(1) 25	(3) 75	(5) 14
2,000–2,500	(1) 6	0	(1) 25	(3) 75	(5) 14
2,500–3,000	0	0	0	(1) 25	(1) 3

Temperature (Fahrenheit)	Disk Not Radially Symmetrical	One or More Rays Dwarfed or Enlarged	Six to Ten Rays	Three Basals	Total
80°–75°	2	3	0	0	5
75 –70	3	4	0	0	7
70 –65	3	3	0	0	6
65 –60	3	3	1	0	7
60 –55	2	2	1	0	5
55 –50	2	2	1	0	5
50 –45	1	1	2	0	4
45 –40	2	2	2	1	7
40 –35	1	2	3	1	7
35 –30	1	1	2	1	5
30 –25	1	0	1	0	2

Systematic Distribution.—

Heliometrinæ
Promachocrinus
Pentametrocrinidæ
Thaumatocrinus
Bourgueticrinidæ
Monachocrinus (part) *Rhizocrinus*

Depth (Fathoms)	Number of Genera with Asymmetrical Disks	Number of Genera with Symmetrical Disks	The Number of Genera having Asymmetrical Disks Expressed as a Percentage of the Number with Symmetrical Disks
0–50	15	51	29
50–100	13	55	23
100–150	11	53	21
150–200	9	45	20
200–250	4	40	10
250–300	4	35	11
300–350	2	31	6
350–400	2	34	6
400–450	2	32	6
450–500	2	30	7
500–550	2	29	7
550–600	2	30	7
600–650	2	29	7
650–700	2	26	8
700–750	2	26	8
750–800	2	22	9
800–850	2	21	9
850–900	1	21	5
900–950	1	23	4
950–1,000	1	20	5
1,000–1,100	1	20	5
1,100–1,200	1	16	5
1,200–1,300	1	13	8
1,300–1,400	1	12	8
1,400–1,500	1	10	10
1,500–1,600	1	10	10
1,600–1,700	1	7	14
1,700–1,800	1	7	14
1,800–1,900	1	6	16
1,900–2,000	1	6	16
2,000–2,500	1	6	16
2,500–3,000	0	4	0

4. *Three Basals*

Geographical Distribution.—Antarctic regions, and northward to northwestern Africa, the Caroline Islands, and British Columbia, except in the antarctic always in very deep water.

Systematic Distribution.—

Plicatocrinidæ

Ptilocrinus	*Gephyrocrinus*
Hyocrinus	*Thalassocrinus*

The frequency of each of these four types of asymmetry at different depths and temperatures is given in the tables on page 541 and in Fig. 4.

Depth (Fathoms)	Number of Genera with Asymmetrical Rays	Number of Genera with Symmetrical Rays	The Number of Genera with Asymmetrical Rays Expressed as a Percentage of the Number with Symmetrical Rays
0–50	7	59	12
50–100	6	62	9
100–150	4	60	6
150–200	2	52	4
200–250	0	44	0
250–300	0	39	0
300–350	0	33	0
350–400	1	35	3
400–450	1	33	3
450–500	1	31	3
500–550	1	30	3
550–600	2	30	6
600–650	1	30	3
650–700	1	27	4
700–750	1	27	4
750–800	1	23	4
800–850	0	23	0
850–900	0	22	0
900–950	1	23	4
950–1,000	0	21	0
1,000–1,100	0	21	0
1,100–1,200	0	17	0
1,200–1,300	0	14	0
1,300–1,400	0	13	0
1,400–1,500	0	11	0
1,500–1,600	0	11	0
1,600–1,700	0	8	0
1,700–1,800	0	8	0
1,800–1,900	0	7	0
1,900–2,000	0	7	0
2,000–2,500	0	7	0
2,500–3,000	0	4	0

In the table showing the frequency at different depths the numbers in parentheses represent the actual cases, the other numbers being the percentage of the total number of genera in which the feature under consideration is found. This last is given in parentheses at the head of each column.

For a graphic representation of the data in the table on the lower part of page 541 see Fig. 5.

These frequencies group themselves as follows:

80°–60°	6.2
60–45	4.7
45–30	6.3
Below 30	2.0

or, segregating those occurring at the optimum temperature:

80°–65°	6.0
65–50	*5.6*
50–30	5.7
Below 30	2.0

Depth (Fathoms)	Number of Genera with More Than Five Rays	Number of Genera with Always Five Rays	The Number of Genera with More Than Five Rays Expressed as a Percentage of the Number with Five Rays
0–50	1	65	1
50–100	2	66	3
100–150	2	62	3
150–200	2	52	4
200–250	2	42	5
250–300	1	38	3
300–350	1	32	3
350–400	2	34	6
400–450	2	32	6
450–500	2	30	6
500–550	2	29	7
550–600	2	30	6
600–650	2	29	7
650–700	3	25	12
700–750	3	25	12
750–800	3	21	14
800–850	3	20	15
850–900	3	19	16
900–950	3	21	14
950–1,000	3	18	16
1,000–1,100	3	18	16
1,100–1,200	3	14	21
1,200–1,300	3	11	27
1,300–1,400	2	11	18
1,400–1,500	2	9	22
1,500–1,600	2	9	22
1,600–1,700	2	6	33
1,700–1,800	2	6	33
1,800–1,900	1	6	16
1,900–2,000	1	6	16
2,000–2,500	1	6	16
2,500–3,000	0	4	0

The relation at different depths between the crinoids in which the disk is not radially symmetrical and those in which it is radially symmetrical is shown in the table on page 542 and in Fig. 6.

The relation at different depths between the crinoids in which one or more rays are dwarfed, or, more rarely, enlarged, and those in which all of the rays are of the same size is shown in the table on page 543 and in Fig. 6.

Depth (Fathoms)	Genera with Three Basals	Genera with Five Basals	The Number of Genera with Three Basals Expressed as a Percentage of the Number with Five Basals
0–50	0	66	0
50–100	0	68	0
100–150	0	64	0
150–200	0	54	0
200–250	0	44	0
250–300	1	38	2
300–350	1	32	3
350–400	1	35	3
400–450	1	33	3
450–500	1	31	3
500–550	1	30	3
550–600	1	31	3
600–650	1	30	3
650–700	1	27	4
700–750	1	27	4
750–800	1	23	4
800–850	1	22	4
850–900	1	21	5
900–950	1	23	4
950–1,000	2	19	10
1,000–1,100	2	19	10
1,100–1,200	2	15	13
1,200–1,300	2	12	16
1,300–1,400	2	11	18
1,400–1,500	2	9	22
1,500–1,600	2	9	22
1,600–1,700	3	5	60
1,700–1,800	3	5	60
1,800–1,900	3	4	75
1,900–2,000	3	4	75
2,000–2,500	3	4	75
2,500–3,000	1	3	33

The relation at different depths between the crinoids with more (less frequently less) than five rays, and those with five rays, is shown in the table on page 544 and in Fig. 6.

The relation at different depths between the crinoids with three basals and those with five is given in the table given above and in Fig. 6.

Summary

Among the recent crinoids any wide departure from the normal close approximation to true pentamerous symmetry indicates unfavorable conditions of one or other of two main types, which are not mutually exclusive.

These two types are

1. Internal unfavorable conditions, induced by incipient phylogenetical degeneration through type-senescence, as in the Plicatocrinidæ, which in the recent seas represent the almost exclusively palæozoic Inadunata; and

2. External unfavorable conditions, taking the form of

> (*a*) *Phylogenetically excessive cold*, which, to cite one example, appears to be the determining factor in the asymmetry of the genus *Promachocrinus;* or of
>
> (*b*) *Phylogenetically excessive warmth*, which appears to be the determining factor in the asymmetry of the family Comasteridæ.

INHERITANCE OF HABIT IN THE COMMON BEAN

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HABIT is the external form of a plant taken as a whole. It is usually described by a few general adjectives, such as erect, open, spreading, etc. However, to study the inheritance of plant habit, a detailed analysis of the real characters underlying habit must be made. It is usually found that the general outer appearance of a plant, its habit, is the result of a combination of independent characters, units, the recombination of which by crossing often results in plants much altered in appearance from the parent varieties. Characters usually unimportant may be found of primary importance in the formation of plant habit.

An example of such inheritance of habit is found in one of Webber's pepper hybrids (6). A cross was made between Red Chili, a variety with many erect fine branches, and Golden Dawn, with few, horizontal, coarse branches, both being of medium size. In the second generation recombination and segregation of the three character pairs occurred, although not in strict Mendelian proportions. The important feature of the results, however, lies in the apparent creation of a giant and a dwarf type, not by the appearance of new units by mutation, but simply by the transference of the characters fine and coarse branches. Hybrids having erect, many and coarse branches were giants, while those having few, horizontal and coarse branches were dwarfs. Other combinations of these characters gave intermediate forms.

The study here reported was made largely on third and fourth generation plants and a few second generation plants of hybrids made primarily for the study of pig-

mentation. The material worked with, owing chiefly to lack of knowledge of earlier generations, offered many limitations and is unsuited to a detailed analysis of the characters in question. As the plants were usually not more than six inches apart in the rows, the crowding in the later stages of development hindered accurate judgment of the habit type.

With reference to general habit bean plants are either pole or bush. Pole beans are commonly long twining vines, climbing when provided with poles or other support. The true bush type is usually short, erect and non-twining. There are also certain races of beans really intermediate between the true bush and pole types, the runner beans, which are non-climbing. Types classed as bush beans also occur, which are spreading and possess outstretched branches of a more or less runner-like character.

The following table contains a description of habit of varieties of beans considered in this discussion. The descriptions are from "American Varieties of Garden Beans" (5). The varieties observed agree with these descriptions except in the case of Mohawk, which is described as without runners. The strain of Mohawk isolated here produces runners.

TABLE I

DESCRIPTION OF BEAN VARIETIES

Pole Beans ALT[1]

Golden Carmine—Small, good climber.
Creasback—Small, at first bush-like, poor climber when young.

Runner Beans ALt

White Marrow—Very large, very spreading, many runners.

Bush Beans AlT

Burpee Stringless—Large, medium, very erect when young, with a few shoots high above the plant, but more or less spreading when mature; no runners.
Giant Stringless—Same as above.

[1] For the meaning of these letters see page 550.

Semi-runner Forms Alt

Refugee—Very large, very spreading, many semi-runners.

Refugee Wax—Large, medium, very spreading, many runner-like branches.

Spreading Forms aLT or *aLt*

Longfellow—Large to medium, somewhat spreading, many outstretched branches, no real runners.

Kenny Rustless—Large, very spreading, almost runner-like branches.

Prolific Black Wax—Medium, more or less spreading, sometimes long outstretched branches, no real runners.

Erect Forms alT or *alt*

Black Valentine—Large, medium, fairly erect, occasional drooping branches, no real runners.

Blue Pod—Medium, erect, no runners or spreading branches.

Bountiful—Large, medium, fairly erect when young, but drooping when mature, no runners or decided spreading branches.

Burpee Kidney—Large, no runners, but usually drooping with fruit-laden branches and spreading when mature.

Burpee White Wax—Large, sometimes with drooping branches, but no real runners.

Challenge Black Wax—Very small, erect, no runners or spreading branches.

Curries—Medium, erect, no runners or spreading branches.

Davis Wax—Large, medium, erect, no runners.

Early Refugee—Medium, very erect, no runners or spreading branches.

German Black Wax—Medium, erect when young, usually borne down with fruit laden branches when mature, no runners.

Long Yellow Six Weeks—Medium, very erect, no runners or spreading branches.

Low Champion—Very large, usually erect, no runners or spreading branches.

Mohawk—Large, very erect, no runners, sometimes drooping when old.

Red Valentine—Medium, erect, no runners or spreading branches.

Round Yellow Six Weeks—Small, medium, very erect, no runners or spreading branches.

Wardwell—Large, medium, fairly erect, no runners.

Warren—Very large, usually erect, no runners or decided spreading branches.

Warwick—Medium, erect, no runners or spreading branches.

R. A. Emerson in his experiments on heredity of plant habit in beans found three main character pairs concerned, namely, length of plant axis, developed in various degrees; twining habit or circumnutation developed in various degrees or not at all; and lastly, the position of pods, axial or terminal. His data involve chiefly the latter character pair, which is inherited in a 3:1 propor-

tion, the axial position of pods being dominant. The position of pods or flowers influences plant habit in this manner: when flowers are formed at the growing tip of a main stem or branch, such a stem or branch must necessarily cease to elongate; on the other hand, if no flowers or fruits are formed at that point it may continue to grow indefinitely.

The habit of all the varieties of beans can be accounted for easily with only these three character pairs. In Table I the varieties here concerned have been grouped according to the probable presence or absence in them of the characters mentioned.

I have designated the axial position of the pods as A, the terminal position by a; long plant axis by L, short by l; a long axis was shown to be dominant over short in some of Mendel's crosses of beans (1). I have designated circumnutation by T and its absence by t, as, judging from Emerson's statements, and according to my own observations twining habit is dominant. The possible combinations of these characters are as follows:

Habit Types

Type *a*,		ALT	Pole beans.
Type *b*,		ALt	Runner beans.
Type *c*,		AlT	Shoots.
Type *d*,		Alt	Semi-runners.
Type *e*,	1	aLT	Spreading with long outstretched branches.
	2	aLt	
Type *f*,	1	alT	Erect bush beans.
	2	alt	

Type *a* comprises the pole beans, as the vines are of great length, both on account of long axis and not being checked by any terminal inflorescence, and as they can climb by virtue of circumnutation.

Type *b* comprises the runner beans. They are like the pole beans except that the climbing habit is not developed to any great extent, if at all. Between these two types it is difficult to draw sharp distinction, but the true runner probably lacks the factor for twining.

Type *c* probably represents the varieties which early send up a few shoots high in the air like Burpee Stringless. In such beans the growth of the main stems or branches is not entirely prevented by the absence of the character which produces a long axis, and as the climbing habit is more or less developed, the characteristic shoots are sent up.

Type *d* represents the semi-runners, caused by the short axis.

Combinations of type *e* and e_2 are the spreading varieties, with long outstretched branches. They are to be distinguished from runners by terminal inflorescences. Kenny Rustless is a representative of the *e* type of habit and probably Prolific Black Wax also.

The last two combinations, *f* and f_2, are the typical erect bush form, such as Blue Pod Butter and Challenge Black Wax.

Table II gives the possible crosses of these types and the F_2 proportions to be expected when the forms crossed are the most nearly typical. In the cases of typical forms, the F_2 types should be differentiated without much difficulty. A circumstance that must be looked upon as a possible cause of exceptions is the presence of unknown factors that cause variations in the intensity of the development of the twining habit and of the intermediate lengths between long and short axis. If there are various factors for length, as Emerson assumes to be the case in all quantitative characters (3), and if the twining habit is to be explained in much the same way, results may be considerably at variance with the expectations indicated in Table II. It must be remembered that the constitutions given for the varieties are only assumed.

At present, owing to circumstances mentioned before,

TABLE II

No.	Constitution	Type	F_2 Proportions
1	ALT × ALT	*a* × *a*	*a*
2	ALT × ALt	*a* × *b*	3*a*: 1*b*
3	ALT × AlT	*a* × *c*	3*a*: 1*c*
4	ALT × Alt	*a* × *d*	

	ALT	gametes	7a					
	ALt		2	3b				
	AlT				3c			
	Alt					1d		
			9a: 3b: 3c: 1d					
5	ALT × aLT	a × e	3a: 1c					
6	ALT × aLt	a × e						
	ALT	gametes	7a					
	ALt		2	3b				
	aLT				$3e_1$			
	aLt				$1e_2$			
			9a: 3b: 4e					
7	ALT × alT	a × f	7a					
	ALT	gametes	2	3b				
	AlT				3e			
	aLT					1f		
	alT		9a: 3b: 3e: 1f					
8	ALT × alt	a × f	15a					
	ALT		6	7b				
	ALt		4		7c			
	AlT		2	2	2	3d		
	Alt						7e	
	aLT						5	
	aLt							3f
	alT							1
	alt		27a: 9b: 9c: 3d: 12e: 4f					
9	ALt × ALt	b × b		b				
10	ALt × AlT	b × c	9a: 3b: 3c: 1d as in type No. 4.					
11	ALt × Alt	b × d		3b: 1d				
12	ALt × aLt	b × e		3b: 1e				
13	ALt × aLT	b × e	7a					
	ALT	gametes	2	3b				
	ALt				3e			
	aLT				1			
	aLt		9	3	4			
14	ALt × alT	b × f	27a: 9b: 9c: 3d: 12e: 4f as in type No. 8.					
15	ALt × alt	b × f						
	ALt	gametes	7b					
	Alt		2	3d				
	aLt				3e			
	alt					1f		
			9b: 3d: 3e: 1f					

16	AlT × AlT	$c \times c$	c
17	AlT × Alt	$c \times d$	$3c: 1d$
18	AlT × aLT	$c \times e$	$9a: 3c: 3e: 1f$ as in type No. 7.
19	AlT × alT	$c \times f$	
20	AlT × aLt	$c \times e$	$3c: 1f$
21	AlT × alt	$c \times f$	$27a: 9b: 9c: 1d: 12e: 4f$ as in type No. 8.
	AlT		
	Alt		$7c$
	alT		$2 \quad 3d$
	alt		$3f$
			1
			$9c: 3d: 4f$
22	Alt × Alt	$d \times d$	d
23	Alt × aLT	$d \times e$	$27a: 9b: 9c: 3d: 12e: 4f$ as in type No. 8.
24	Alt × aLt	$d \times e$	
	ALt		$7b$
	Alt		$2 \quad 3d$
	aLt gametes		$3e$
	alt		$1f$
			$9b: 3d: 3e: 1f$
25	Alt × alT	$d \times f$	$7c$
	AlT		$2 \quad 3d$
	Alt		$3f$
	alT gametes		1
	alt		$9c: 3d: 4f$
26	Alt × alt	$d \times f$	$3d: 1f$
27	aLT × aLT	$e \times e$	e
28	aLT × aLt	$e \times e$	e
29	aLT × alT	$e \times f$	$3e \times 1f$
30	aLT × alt	$e \times f$	
	aLT		$7e$
	aLt		5
	alT gametes		$3f$
	alt		$1f$
			$12e: 4f$
			$3 : 1$
31	aLt × aLt	$e \times e$	e
32	aLt × alT	$e \times f$	$3e: 1f$ as in type 30.
33	aLt × alt	$e \times f$	$3e: 1f$
34	alT × alT	$f \times f$	f
35	alT × alt	$f \times f$	f
36	alt × alt	$f \times f$	f

only general notes on the behavior of various types of crosses can be given.

Type 2. ALT × ALt

In the third generation of a cross of Creasback, a typical pole bean with White Marrow, a runner bean with probably a weak character for circumnutation, all lots were of axillary inflorescence. The habit of climbing was developed in various degrees so that classifications of types was difficult.

Cross Type 6 or 7. ALT + aLT or aLt

Notes on an early cross of Creasback by Prolific Black Wax indicate that the generation F_1 were pole beans, the generation F_2 segregating into 33 pole and 8 bush. The latter is probably a 3:1 proportion as expected. Whether all plants described as bush were of the spreading type does not appear from our records.

Cross Type 8. ALT × *alt* or alT

In a cross of Creasback with Blue Pod, a typical bush bean, there occurs one strain of homozygous pole plants, and also in the F_4 generation heterozygous types. Pole and runner forms and bush forms of various types occur in the proportions of 9:7 in one lot and in another of 3:1, as might be expected in an F_4 generation. In another small lot occur plants with long outstretched branches, in another two plants of *c* type of habit. Evidently Blue Pod has the constitution alt.

The date from a cross of Creasback with Blue Pod do not signify much, as the types isolated happen to be constant, one a pole type and bush types, of which several are described as somewhat spreading. In one there occurs a runner bean.

Creasback and Warwick crosses in the F_4 generation behave consistently with the cross type, as assumed. In one lot, 1 2 have axial inflorescence and three terminal. Lots with spreading plants occur and one plant was noted which possessed a very long axis, along with a twining habit, but also terminal inflorescence. According to the

explanation of habit characters assumed, such a plant would have the formula aLT. Without a support which happened to have been placed near it, the peculiarity of the plant would not have been so noticeable.

A cross of Mohawk and Golden Carmine, a pole bean, gave in the F_2 generation 7 plants of the bush type and 28 plants more or less pole like. In the notes no separation of pole and runner beans were made, probably due to a lack of clear distinction between the two as occurs in many crosses.

Cross Type 10. ALt × AlT

White Marrow by Burpee Stringless is presumably a cross of this type. In one case the F is described as a pole and in another as a runner bean. The F_2 generation results in 38 bush to 108 described as runner beans. This is consistent with expected results when the plant is described as a whole. The expectations are 12 pole and more or less pole like beans and four more or less bush like forms.

Cross Type 12 or 13. ALt + aLT or aLt

A cross of White Marrow, a runner variety, with Prolific Black Wax, which belongs to the type with spreading outstretched branches, gave 20 bush plants and 58 plants of the runner and pole types, no differentiation being made between the two. This is consistent with the assumed constitutions.

Cross Type 14 or 15. ALt × alt or alT

White Marrow with Currie behaves according to expectation, giving in the F_2 generation 41 bush plants *e* or *f* in type, and 52 of the runner or semi-runner type.

In the cross of Blue Pod by White Marrow and its reciprocal, neither variety being pole in type, climbing plants apparently occur as well representative of most if not all of the other habit types. Some lots isolated were very erect, others spreading in various degrees; one lot is de-

scribed as having long tendril-like shoots above the plant, another along side of this had shorter shoots, perhaps AlT. Among the lots, all degrees of climbing were developed; one plant encountered was evidently aLT like the one mentioned in a previously discussed cross; plants with more or less outstretched branches were noted. Type notes on F_1 and F_2 generations of an earlier cross in type; F_2 segregates into 25 bush forms and 62 runners, are significant. The F_1 generation is described as pole probably including pole beans of the F_1 type. The ratio is disturbed by the lack of a clear understanding of the true basis for classification of plant type in beans. The F_2 of another cross involving the same varieties is noted as having 41 bush and 5 runner beans.

White Marrow and Burpee Kidney yielded two lots of bush beans and two heterozygote lots giving 6 plants with terminal inflorescence and 15 with axillary.

Red Valentine and White Marrow crosses give similar results. In an early cross, the F_2 generation plants have been grouped according to the general plant type, no attempt being made to separate intergrading types. The notes give the results of segregations as 75 bush and 136 runner beans. Later generation heterozygotes approach a proportion of 9 runner to 7 bush beans. The apparent behavior probably depends on whether the intermediate types are classed as runner or bush. In the cross in which only the F_4 generation was observed, only constant bush types seem to have been isolated.

Cross Type 19 or 21. AlT × alt or alT

Blue Pod crossed with Burpee is a representative cross of this type. Only in a few cases was the Burpee type, plants with shoots high in the air, observed, as most lots isolated were homozygous and erect. In the F_2 generation of an early cross, plants described as runners appeared. The proportion was 3 runners to 1 bush. Heterozygote lots descended from these plants segregated in the same manner, totaled 18 bush and 71 so-called run-

ners. The runners are probably really *c* in type or *c* and *d*.

In the cross of Giant Stringless and Blue Pod the parent types were both isolated. No semi-runners were noted, as would be the case if the cross were No. 21 in type.

CROSS TYPE 25 OR 26. Alt × aLT OR alt

Refugee Wax is a semi-runner bean. The F_3 isolated lots of this variety crossed with Blue Pod were all more or less erect. Some lots homozygous for axial branching were isolated, many individuals of which showed signs of climbing. The semi-running and climbing branches were short, confirming the assumption that neither variety used possesses the factor for a long axis. The climbing tendency exhibited shows that there must be strains of Blue Pod that possess T. Previous data are in harmony with this.

CROSS TYPE 29, 30, 34 OR 35. aLT OR aLt × *alt* OR alT

Many crosses of bush beans with those of spreading type give a 3:1 proportion in the F_2 and later heterozygous lots.

In Keeny Rustless, a variety of the spreading type, with its almost runner-like branches, by Red Valentine some lots with the spreading habit have been isolated, also more or less runner-like forms and one with the erect habit of Red Valentine. The axial and terminal inflorescence is inherited in a 3:1 proportion. Notes on type in one heterozygous lot show five erect and 10 plants with outstretched branches.

In the cross of Black Valentine and Prolific Black Wax one lot with outstretched branches was isolated; all others were of the erect type.

In the cross of Blue Pod Butter and Prolific Black Wax no spreading types with outstretched branches were noted, but this is not surprising, as in an F_4 generation the parent plants selfed for planting may not have hap-

pened to be of the spreading type, thus giving homozygous erect offspring.

In the cross of Golden Eyed Wax with Prolific Black, outstretched branches due only to axial inflorescence were noted.

Spreading plants of this nature also occur in the cross of Bountiful and Prolific Black Wax. In the latter two crosses the twining habit was more or less developed in the longer branches.

Cross Type 34, 35, or 36. alT × alT, alT × alT, or alt × alt

In the crosses of this type only erect bush beans without runners or spreading branches, should occur, although contorted stems might possibly appear. Such is the behavior of the following crosses of this type:

Low Champion × Blue Pod Butter
Blue Pod Butter × Golden Eyed Wax and reciprocal
Blue Pod Butter × Mohawk and reciprocal
Challenge Black Wax × Warwick
Currie × Mohawk and reciprocal
Currie × Red Valentine
Blue Pod Butter × Warren
Bountiful × German Black Wax

In the crosses, Challenge Black Wax by Davis Wax and Blue Pod Butter by Davis Wax, lots have been isolated with short shoots above the plants somewhat resembling the habit of Burpee Stringless and Giant Stringless. This behavior is unexpected if such a plant type is to be described by the formula AlT. The Davis Wax type used in the crosses may, however, have been of a different strain from that described in the table. This variety is the only one used in the crosses that was not under the observation of the writer, as its growth was discontinued the year in which these notes were taken.

While the factors discussed above primarily determine the plant habit, there are several others of secondary consideration. No special notes were taken with regard to

these. Some of them are mentioned in the following paragraph.

The character of the habit type is somewhat influenced by the amount of branching the plants exhibit; open, loose, bush beans are the result of few branches; the close, dense habit of some forms is caused by profuse branching. The size of a plant to some extent influences the habit, although not as much in small ones like Challenge Black Wax. In Warren the size of the plant probably causes it to droop. In some varieties the number and weight of the pods, as well as their position, cause some plants to droop and assume a spreading habit when old. Perhaps fineness and coarseness of branching affect habit.

One further matter that comes up for consideration is the question of the effect of environment upon plant habit. Its greatest effect, as would be supposed, seems to be upon such quantitative characters as length of the plant axis and probably the twining character to some extent. Instances of adverse conditions resulting in the almost total suppression of a character were noted in plants grown on poor soil. They exhibited the slender tips, typical of vines with axial inflorescence, but were otherwise bush-like and erect. The accelerating effects of very fertile soil on the growth of runner was also noted. However, the environmental explanation for the sudden appearance of runners among bush beans or of pole beans among typical runners is open to question. The most probable cause of such phenomena lies primarily in the regrouping of the unit characters of habit, combined at times with checking and accelerating factors external to the plant.

The investigations here reported offer a foundation upon which more extensive study on the subject might be based.

The following table suggests a few important cross types and the varieties which might be used to advantage:

Crosses for Further Study

No.	Type Number	Varieties		Plant Type
1	2	Golden Carmine	× White Marrow and reciprocal	*a* × *b*
2	3	Golden Carmine	× Burpee Stringless and reciprocal	*a* × *c*
3	4	Golden Carmine	× Refugee and reciprocal	*a* × *d*
4	5 or 6	Golden Carmine	× Keeney and reciprocal	*a* × *e*
5	7 or 8	Golden Carmine	× Challenge Black and reciprocal	*a* × *f*
6	10	White Marrow	× Burpee Stringless and reciprocal	*b* × *c*
7	11	White Marrow	× Refugee and reciprocal	*b* × *d*
8	12 or 13	White Marrow	× Keeney and reciprocal	*b* × *e*
9	14 or 15	White Marrow	× Challenge Black and reciprocal	*b* × *f*
10	17	Burpee Stringless	× Refugee and reciprocal	*c* × *d*
11	18 or 19	Burpee Stringless	× Keeney and reciprocal	*c* × *e*
12	20 or 21	Burpee Stringless	× Challege Black and reciprocal	*c* × *f*
13	23 or 24	Refugee	× Keeney and reciprocal	*d* × *e*
14	25 or 26	Refugee	× Challenge Black and reciprocal	*d* × *f*
15	29 or 30	Keeney	× Challenge Black and reciprocal	*e* × *f*

The Burpee crosses should be particularly watched to determine if the assumed set of factors AlT is the cause of the shoots and later spreading habit of the plant.

The axis should be studied by means of accurate measurement as far as possible. The judgment concerning circumnutation would probably be necessarily more or less indefinite.

In crosses 4, 5, 8, 9, 11, etc. the type number should be determined.

The conclusions that can be drawn from observations reported in the preceding pages are:

1. That plant habit in beans is largely determined by the presence or absence of three characters which have been designated by the letters A, L, and T.

1. A, the presence of axial inflorescence permitting an indefinite growth, of the main stem and main branches, and a terminal inflorescence causing definite growth.

2. The length of the axis L, an important factor controlling plant habit and probably governed by a series of two or more factors for a length L_1, L_2, etc., which behave after the fashion of Emerson's hypothesis for the inheritance of quantitative characters.

3. The climbing habit is due to a factor for circum-

nutation. This factor may be called T. The cause of the various degrees of the climbing habit has not been determined with any degree of certainty. The contorted stems of erect bush forms are probably caused by T.

II. The factors A, L and T may be present in any possible combination, giving rise to the various habit types of beans.

III. When the types are crossed among themselves they behave approximately after the manner sketched in Table II.

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ON THE MODIFICATION OF CHARACTERS BY CROSSING[1]

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In the early years of Mendelian discovery there was much discussion concerning gametic purity in hybrids, and the question whether unit characters are modified on crossing was keenly debated. Convinced by the numerous instances in which Mendelian characters appear to be unmodified by crossing, many writers came to the conclusion that characters universally segregate without being modified or "contaminated" by association with other characters in the hybrid. That such a conclusion is far too sweeping is, however, indicated by many later results, and there is now a disposition to admit that changes in a character or the breaking up of a character may be effected through crossing. But some writers continue to look upon a unit character as an entity, which is unmodifiable and indestructible by hybridization.

Notwithstanding the admitted belief of Bateson and others that characters may be modified by crossing, I know of no extensive body of evidence that such modifications take place except the work of Castle and Phillips (1914) whose conclusions have not been fully accepted and are chiefly concerned with modification by selection. It therefore seemed worth while to direct attention to certain experimental results of a somewhat different kind which appear to show beyond cavil that modifications of characters sometimes result from crossing. The matter is an important one because it affects the old question of the swamping of new characters through crossing, as well as various other aspects of evolutionary theory.

[1] Read before the American Genetic Association, San Francisco meeting, August 3, 1915.

Anticipating the conclusions which will be reached in this paper, it may be pointed out that the swamping effect is not so serious a check upon progressive evolution as might be supposed, (1) because blending or modification of a new character only takes place in certain crosses and may be accompanied by segregation even in some of those, and (2) because Mendelian characters usually come out "pure" when crossed with the form from which they were derived. Hence when Mendelian characters arise through mutations in nature it may be expected that they will be able to perpetuate themselves and spread, especially when dominant, unless they place the organism at a disadvantage in the struggle for existence. The modification of a Mendelian character will come, not from crossing with its parent form but with a more distantly related species.

Some writers appear to believe that it is practically impossible to modify a unit character because it is represented in the germ plasm by a "gene" whose essential characteristic is its unmodifiability. But if we consider that each unit character is a *difference* which has arisen through a change in one element of the germ plasm, probably in a chromosome, then it would seem possible that if introduced into a foreign cytoplasm the chromosome may become subject to permanent modification.

Castle and Phillips (1914) have produced evidence from hooded rats tending to show that selection may modify a unit character in certain cases, although the nature of this result is not yet fully analyzed. They moreover show that the hooded character is modified by a cross. Davenport (1906) in his experiments with poultry, concluded that unit characters are frequently modified by crossing. He says (p. 80):

> Very frequently, if not always, the character that has been once crossed has been affected by its opposite with which it was mated and whose place it has taken in the hybrid. It may be extracted therefrom to use in a new combination, but it will be found to be altered. This we

have seen to be true for almost every characteristic sufficiently studied—for the comb form, the nostril form, cerebral hernia, crest, muff, tail length, vulture hock, foot feathering, foot color, earlobe and both general and special plumage color. Everywhere unit characters are changed by hybridizing.

In crosses between *Œnothera rubricalyx* and *Œ. grandiflora* I have studied with care the modifications which take place in the expression of the various character-differences in F_1, F_2 and later generations. Many of the results have been recorded in detail elsewhere (Gates, 1914, 1915*a*, pp. 250–282). It need only be said that the foliage characters in F_2 form an absolutely continuous series so that it is impossible to apply to them usefully the unit-character conception. In F_3 a large number of races were obtained differing in many ways as regards their foliage, many of them breeding true and others varying within wide or narrow limits. Occasionally in back-crosses an apparently complete reversion takes place to one or other of the parents, but blending and fractionation of the characters is the rule.

It is, however, difficult to obtain critical evidence from the foliage because, while the original differences are sharply marked, yet it is always possible to assume that the continuous F_2 series and the numerous F_3 races result from the presence of many independent units.[2] I will therefore confine my attention to the sharp pigmentation character (R) of *rubricalyx*, for in the inheritance of this character crucial evidence may be obtained. The origin of this dominant unit-character through a single mutation, and the subsequent attainment of the duplicate condition (RR′) for this character in some of the offspring of later generations (1915*b*), have been pointed out elsewhere. Here we will examine the modifications of R which take place when *rubricalyx* is crossed with *Œ. grandiflora*.

The main facts regarding the variability of R in these

[2] The inheritance of pubescence-differences shows similar features and can not be reasonably interpreted in terms of numerous units.

crosses have already been published (Gates, 1914, p. 244 and 1915*a*, p. 257) and need only be summarized here, to emphasize their significance. In the publications cited I had not yet recognized that the occurrence of 15:1 ratios in later generations of *rubricalyx* is significant as indicating that in such families the duplicate condition for R had been reached, even although other ratios such as 5:1 occur as well.

The F_2 generation of the crosses between *rubricalyx* and *grandiflora* contained 2,794 plants, in 20 of which the red bud-character R showed decided modification so as to be more or less intermediate between the two parents. Since each plant in bloom produces scores of buds simultaneously, and hundreds during the season, there is ample material for determining the exact degree of modification or development of the character in every individual. As will be seen from the original records, the 20 plants in which the color pattern was more or less modified were not all alike but formed a series, some being nearer the normal R than others. In most other F_2 plants sharp segregation took place, the buds being entirely either R or r without the slightest doubt in classification. In addition to the 20 plants above mentioned, there were, however, a certain number in which the character R was more or less underdeveloped, so that it was impossible to be certain whether they represented mere fluctuations or real modifications of the character.

The crucial test of modification is supplied by the F_3 generation. Two of these last-mentioned intermediate plants self-pollinated yielded offspring like themselves, without any tendency to segregate into the R and r types. These families numbered, respectively, 283 and 20 plants, so that in the former case at least any tendency to segregation could not fail to be observed. The buds of these plants were intermediate, the pigmentation was pale and was never fully developed on the hypanthium as is the case in *rubricalyx*. The whole population was then intermediate like the parent.

Another F_3 family (No. 149) was derived from an F_2 plant (65. III. 12) having sepals weak red with the color pattern as extensive as in *rubrinervis* 6 (*i. e.*, nearly the extreme condition), and in addition streaks of pale red on the hypanthium. This plant was therefore nearer r than R, and one may account for its occurrence through "contamination" before segregation took place in the germ cells of the previous generation. In pure *rubrinervis* or *grandiflora* I have never found even a *trace* of red on the hypanthium until the flower fades. The offspring of this plant numbered 186 individuals and their pigmentation fluctuated about that of the parent plant as a mean. This condition closely approximated that in *Œ. rubrinervoides* (1915*c*, p. 390), which may have originated in a similar way.

We must, therefore, conclude that plants which are intermediate in pigmentation breed true, at least in all cases tested, and that the degree of pigmentation in the parent is adhered to in the offspring whether the parent plant is an under-pigmented R or an over-pigmented r. In this aspect, the inheritance in such cases is quantitative and the offspring vary only within narrow limits.

The quantitative aspect is further emphasized when F_1 and F_2 hybrids of *Œ. grandiflora* and *Œ. rubricalyx* are crossed back with either parent. The pigmentation is much intensified when crossed back with *rubricalyx*, and greatly diluted when crossed with *grandiflora*. Thus in (*rubricalyx* × *grandiflora*) × *grandiflora* if the female parent is heterozygous for R, segregation into R and r plants will occur in the offspring, but the R plants will be much paler than in the selfed offspring of the female parent.

Hence there are two somewhat antagonistic effects which have to be considered, (1) the segregation of R and r individuals, and (2) a permanent dilution of the pigmentation of the R individuals. The former effect can be explained by the meiotic mechanism which segregates

chromosome pairs. The latter effect may be due to a modification of the chromosomes themselves, or perhaps of the surrounding cytoplasm, or the inhibition in pigmentation may be explained by the presence of more numerous *grandiflora* chromosomes. Everywhere, in an accurate study of the inheritance of R, the quantitative as well as the qualitative (presence or absence) aspect has to be considered.

The dilution effect from crossing back with *grandiflora* has been tested in six families numbering 673 individuals and is always essentially the same. Although segregation into the R and r types takes place when the parent is heterozygous, yet R once diluted always remains so and apparently never gives rise to the original deeply pigmented condition. In other words, a permanently blended condition arises as regards the depth of pigmentation, although this will still segregate from the unpigmented condition in heterozygous plants.

It is not easy to furnish a complete explanation for this diluting effect. The permanent dilution of R through union with a *grandiflora* germ cell may perhaps be accounted for by the fact that in the heterozygote the chromosomes of *grandiflora* are closely associated in the same nucleus with those of the other parent. The chromosomes which are finally dissociated in the germ cells, after thousands or millions of mitotic divisions in association, might then be supposed to be somewhat modified. There are, however, difficulties with this view, since the absence-character, r, is usually not contaminated, but splits out sharply and almost invariably without any trace of red-production.

It is also difficult to account for the facts on the assumption that the cytoplasm has been permanently modified.

There is, however, one hypothesis which appears to meet the case. If all the *grandiflora* chromosomes are equally effective in inhibiting anthocyanin production in

the hybrids with *rubricalyx*—a not improbable hypothesis—then the dilution effect will be the same in F_1 or in crossing back, whenever an R chromosome is present in the next generation; and when such a chromosome is not present there will of course be complete absence from the buds of the *rubricalyx* pigment. On this hypothesis, in an original cross between *rubricalyx* and *grandiflora* a certain (observed) reduction in pigmentation occurs. When the F_1 hybrid is crossed back with *grandiflora* the additional *grandiflora* chromosomes thus introduced dilute or inhibit the color still further, while the presence or absence of the diluted R will depend upon whether or not the R chromosome from *rubricalyx* is present. It would thus appear to be unnecessary to assume that this chromosome is itself modified by its different nuclear and cytoplasmic environment.

In other words, the *grandiflora* chromosomes may be supposed to exert a mass effect in inhibiting the influence of the R chromosome. It is, of course, possible that in these circumstances the R chromosome itself may be permanently modified, but it seems possible to explain all the facts without making this assumption. In any case, whatever the *modus operandi,* there can be no question that the R character is permanently diluted by crossing with *grandiflora,* and the degree of dilution is increased every time the hybrid is again crossed back with that species.

Another noteworthy fact is that as the pigmentation becomes more dilute its morphological expression is more irregular. The color pattern of the bud begins to break up, and instead of continuous pigmentation of the whole bud a patchy effect will be produced. This spotted condition of the buds is very marked in certain families, *e. g.,* in the second generation of offspring from (*rubricalyx* × *grandiflora*) × *grandiflora* (see Gates, 1915*a*, Fig. 113, p. 280). When it appears it is found to persist in later generations. To account for this condition through the

accession of a "spotting factor" is a gratuitous assumption. Spotting appears rather to be the manner of expression of the character when the amount of pigment is small. It must be said, however, that in some families having no greater quantity of pigmentation there is a strong tendency for it to remain uniformly distributed, so that the whole bud is very pale red.

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SHORTER ARTICLES AND DISCUSSION

STUDIES ON INBREEDING. VI. SOME FURTHER CONSIDERATIONS REGARDING COUSIN AND RELATED KINDS OF MATING[1]

In the first of these studies[2] the writer dealt with the results, in so far as concerned coefficients of inbreeding, which would follow continued brother × sister, parent × offspring, and cousin × cousin mating. Regarding matings of the latter type it is desired now to record certain further facts.

PEDIGREE TABLE I (HYPOTHETICAL)

To Illustrate the Continued Breeding of First-Cousin × First-Cousin — Single Cousins

Δ	*a*	*c*	*g*	*m*	1, 2
				n	3, 4
			h	*o*	1, 2
				p	5, 6
		e	*i*	*m*	1, 2
				n	3, 4
			j	*q*	1, 2
				r	9, 10
	b	*d*	*g*	*m*	1, 2
				n	3, 4
			h	*o*	1, 2
				p	5, 6
		f	*k*	*m*	1, 2
				n	3, 4
			l	*u*	1, 2
				v	7, 8
Generation number	1	2	3	4	5

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station No. 85.

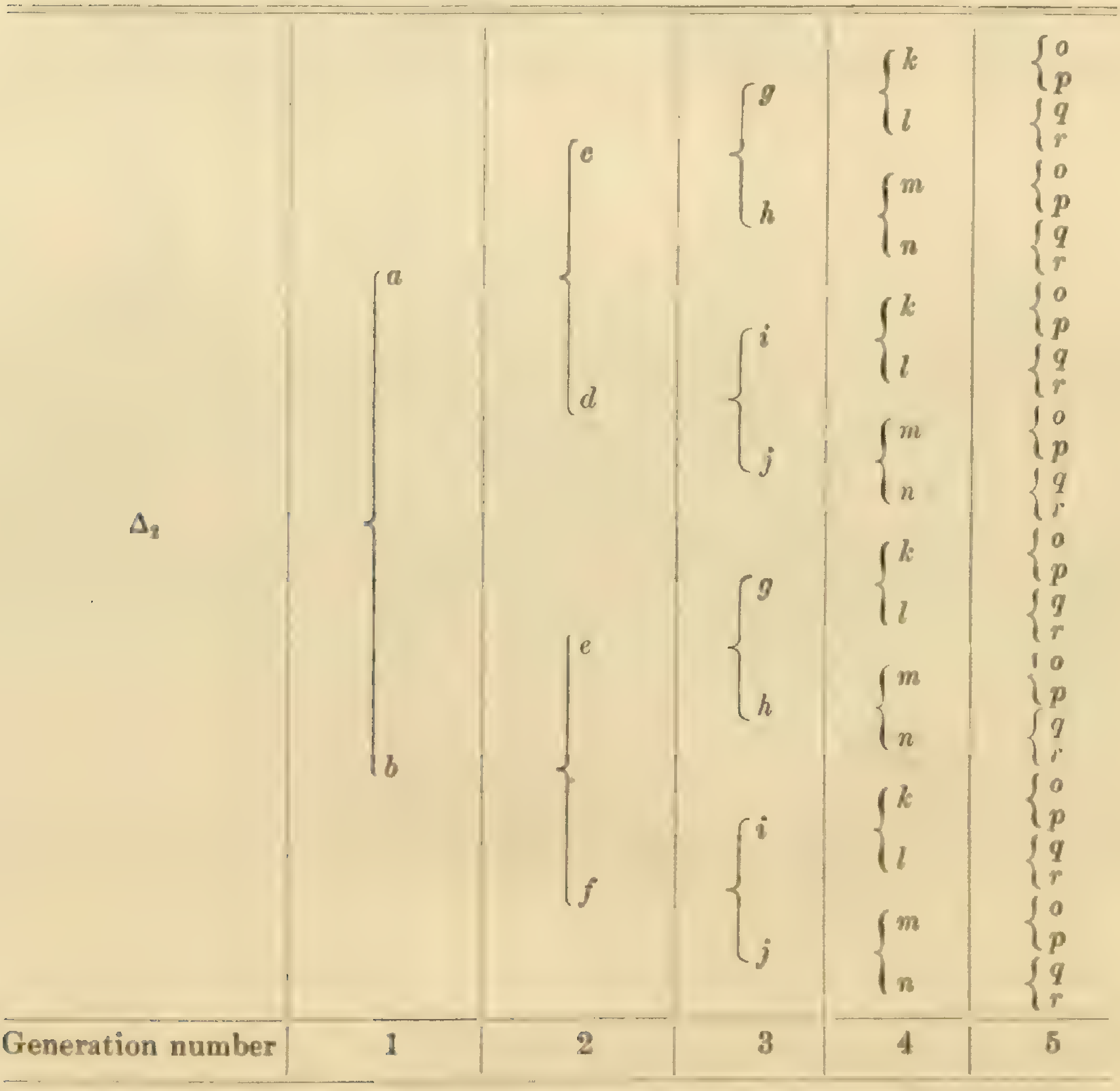
[2] Amer. Nat., Vol. XLVIII, 1913, pp. 577–614.

There are, of course, two possible sorts of first cousins, single and double. In the first case one of the parents of any individual is a brother (or sister) to the one of the parents of the other individual in the mating. In the second case, both the parents occupy this relation to the parents of the other individual in the mating.

These two sorts of first cousinship are shown in Pedigree Tables I and II.

PEDIGREE TABLE II (HYPOTHETICAL)

To Illustrate the Continued Breeding of First-Cousin × First-Cousin — Double Cousins



The values of the coefficients of inbreeding for continued single and double cousin mating are shown in Table I.

It will be seen that Pedigree Table I and the third column of Table I are different from the corresponding values given on pages 591 and 592 of the earlier paper. The present values should be substituted for the earlier ones, which were based upon

the erroneous assumption that half the double-cousin values would give single-cousin values.

TABLE I

VALUES OF THE SUCCESSIVE COEFFICIENTS OF INBREEDING IN THE CASE OF CONTINUED COUSIN MATING

Coefficient of Inbreeding	Ancestral Generation Included	Coefficient for Single Cousins	Coefficient for Double Cousins
Z_0	1	0	0
Z_1	2	0	0
Z_2	3	25.00	50.00
Z_3	4	50.00	75.00
Z_4	5	68.75	87.50
Z_5	6	81.25	93.75
Z_6	7	89.06	96.98
Z_7	8	93.75	98.44
Z_8	9	96.48	99.22
Z_9	10	98.05	99.61
Z_{10}	11	98.93	99.80
Z_{11}	12	99.41	99.90
Z_{12}	13	99.68	99.95
Z_{13}	14	99.83	99.98
Z_{14}	15	99.91	99.99
Z_{15}	16	99.95	99.994

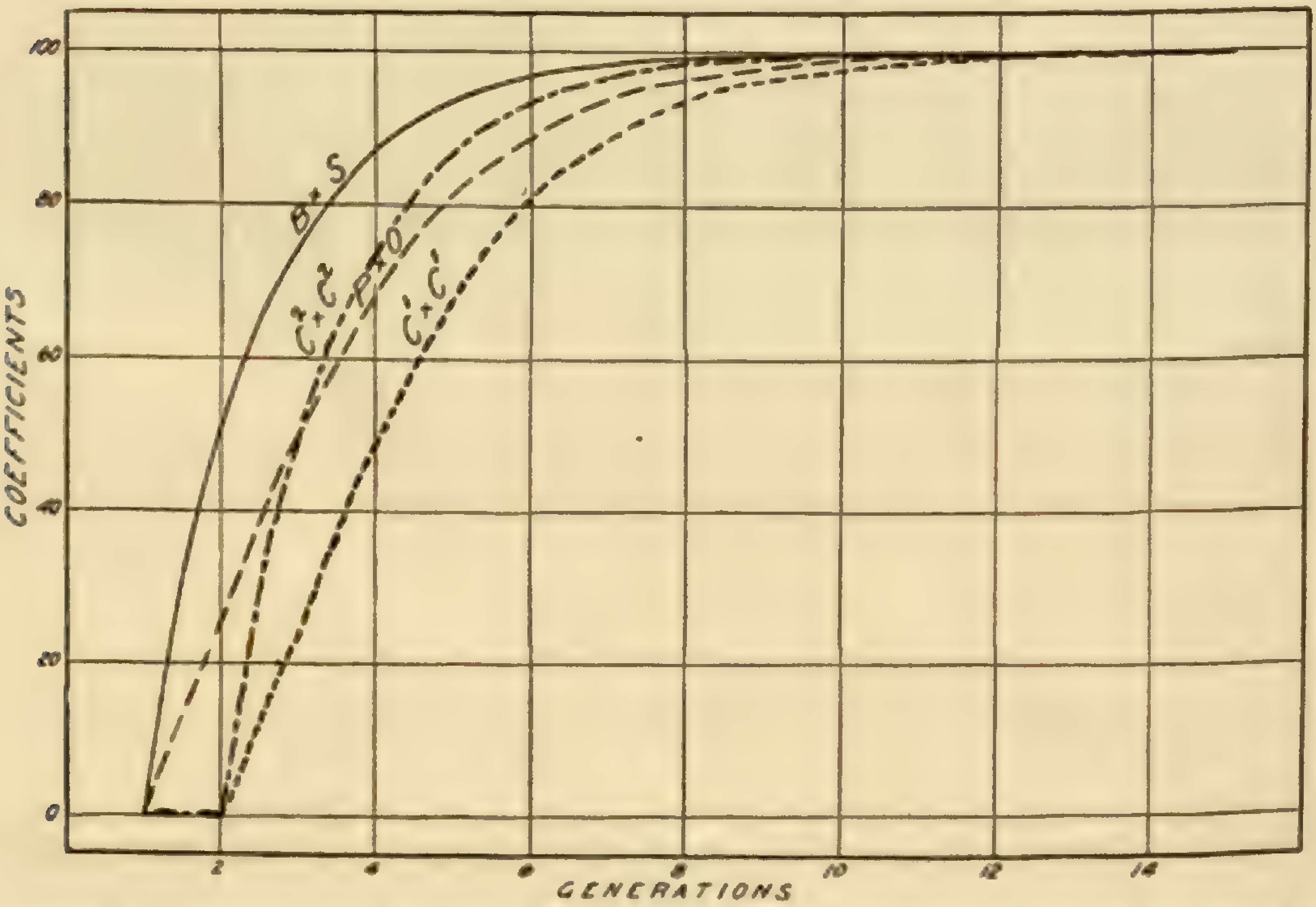


FIG. 1. Curves of inbreeding, showing (*a*) the limiting case of continued brother × sister breeding, wherein the successive coefficients of inbreeding have the maximum values; (*b*) continued parent × offspring mating; (*c*) continued first-cousin × first-cousin mating where the cousinship is double ($C^2 \times C^2$), and (*d*) continued first-cousin × first-cousin mating where the cousinship is single ($C^1 \times C^1$). The continued mating of uncle × niece gives the same curve as $C^1 \times C^1$.

The data of Table I are given graphically in Fig. 1, together with the curve for brother × sister and parent × offspring.

From the table and figure it is seen that with continued inbreeding according to any one of these four types the coefficient approaches the value 100. The rate of approach is different, however, in the different cases. The curves fall into two pairs. The brother × sister and the double cousin curves are precisely alike so far as concerns their curvature or shape at any given point. Similarly, the parent × offspring and single cousin curves are of the same shape. *The essential point of difference is that the cousin curves lag a generation behind the others.*

Let us now consider the question of the degree of inbreeding following continued matings of the avuncular type of relationship. Pedigree Table III gives a pedigree in which each mating is of uncle × niece.

PEDIGREE TABLE III (HYPOTHETICAL)

TO ILLUSTRATE THE MATING OF UNCLE × NIECE

Generation number	1	2	3	4	5
X	*a*	*c*	*g*	*m*	*u*
					v
				n	*w*
					y
			h	*o*	*m*
					n
				p	*z*
					1
		d	*i*	*g*	*m*
					n
				h	*o*
					p
			j	*q*	*g*
					h
				r	2
					3
	b	*e*	*c*	*g*	*m*
					n
				h	*o*
					p
			d	*i*	*g*
					h
				j	*q*
					r
		f	*k*	*c*	*g*
					h
				d	*i*
					j
			l	*s*	*c*
					d
				t	4
					5

From this table it appears that the values of the coefficients of inbreeding will be exactly the same for this type of mating as in the case of single cousin mating. Or, in other words, Z's form the following series.

TABLE II

VALUES OF COEFFICIENTS OF INBREEDING FOR CONTINUED UNCLE × NIECE MATING

Coefficient	Number of Ancestral Generations	Value of Coefficient
Z_0	1	0
Z_1	2	0
Z_2	3	25.00
Z_3	4	50.00
Z_4	5	68.75
Z_5	6	81.25
etc.	etc.	etc. as in Table I

From the data presented in this and former papers it is clear that inbreeding continued for about ten generations, quite regardless of the *type* of mating, provided only it be *continuously* followed, leads to within one or two per cent. of complete "concentration of blood." The bearing of this result upon the general question of the degree of inbreeding which exists in the ancestry of our domestic animals to-day is obvious. To consider but a single case: In 1789[3] a law was passed prohibiting the importation of cattle into the Island of Jersey. Hence it follows that all pure-bred Jersey cattle of the present time must be of the descendants of the relatively few animals on the Island in 1790. Taking three years as about the average generation interval in cattle, this means about forty generations since the Island was closed to importation. The concentration of lines of descent which must have occurred in this time merely by the dropping of lines and quite regardless of the type of mating is obvious. This is not the place to go in detail into the discussion of inbreeding in Jerseys, especially as I hope shortly to publish the results of an extensive study of this matter, but it seems desirable to emphasize the bearing of such hypothetical pedigrees for particular types of mating as are given in this and earlier papers, on the general problem of inbreeding.

It is possible to extend now somewhat the table of general equations given by Jennings[4] for coefficients of inbreeding after

[3] *Teste* Rees's Encyclopedia and H. S. Redfield, *Natl. Stockman and Farmer*, December 15, 1892.

[4] AMER. NAT., Vol. XLIII, p. 695, 1914.

n generations of each particular type of mating. We have the following values, where n denotes the number of ancestral generations concerned, or, as Jennings puts it, the number of successive inbreedings which have taken place.

Type of Mating	Coefficient of Inbreeding
Self-fertilization	$\frac{2^n - 1}{2^n}$
Brother × sister	$\frac{2^n - 2}{2^n}$
Cousin × cousin, single	$\frac{2^n - 2n}{2^n}$
Cousin × cousin, double	$\frac{2^n - 2^2}{2^n}$ $\begin{cases} \text{from } n = 2 \\ \text{to } n = \infty \end{cases}$
Parent × offspring	$\frac{2^n - n - 1}{2^n}$
Uncle × niece	$\frac{2^n - 2n}{2^n}$

RAYMOND PEARL

AN ATTEMPT TO PRODUCE MUTATIONS THROUGH HYBRIDIZATION

THERE is no more interesting problem to the experimental evolutionist than the one relating to the cause or causes of the origin of mutations. Until we are able to solve this problem we can only accept what the gods give in our breeding experiments. When a mutation arises it is usually a simple process to produce a pure stock. By mutation is meant any deviation from the normal type which reappears in some of the descendants. In the following experiment most of the abnormalities that were found never reappeared in the offspring.

My experiments have been confined to the fruit fly, *Drosophila ampelophila*, a species kept for years "under cultivation" at Columbia University. This species has proved to be very plastic, throwing off great numbers of mutant forms. At the suggestion of Dr. T. H. Morgan I crossed some of these mutants with wild stock of the same species from widely separated localities in order to test whether through hybridization mutations arise in greater numbers than in inbred stock.

The idea that new forms arise from crossing more or less closely related species is an old one. One finds many references in Darwin's works to this conception. For instance, in the "Origin of Species" Darwin says:

When mongrels and the more fertile hybrids are propagated for several generations, an extreme amount of variability in the offspring in both cases is notorious; but some few instances of both hybrids and mongrels long retaining a uniform character could be given. The variability, however, in the successive generations of mongrels is, perhaps, greater than in hybrids.

One of the causes of ordinary variability . . . is . . . that the reproductive system from being eminently sensitive to changed conditions of life, fails under these circumstances to perform its proper function of producing offspring closely similar in all respects to the parent form.

From "Plants and Animals under Domestication" we find the following.

Crossing, like any other change in the conditions of life, seems to be an element, probably a potent one, in causing variability.

A variation to be effective in species formation must reappear in some of the descendants. That a variation could, through selection within a *pure strain* be increased or decreased in the direction of selection to form a stable species has been seriously questioned since Johannsen's classic experiments. It is well understood, on the other hand, how selection in a *mixed population* could cause the variation to move in the direction of selection up to a certain point.

The first mutant stock selected for the experiment was cherry club vermilion. The factors for these three characters are linked together and are also linked with sex; the second stock was black pink bent, which has the three factors independent of each other and none is linked with sex. These factors are supposed to lie in the second, third and fourth chromosomes, respectively. The third stock was black purple vestigial arc speck, which has the five factors linked together. They lie in the second chromosome. A stock from France was crossed to the mutant stock several months after the other crosses were made, and eosin tan vermilion was substituted for the cherry club vermilion, and pink kidney sooty rough for the black purple vestigial arc speck stock because flies of these particular stocks were not to be had at the time desired.

These forms were chosen because it was thought that if mutations do arise from hybrid forms there would be more probability of their origin from a mutant varying in several characters when crossed to wild than if it varied in only one character. Also by using stock containing several recessive characters a check could

be placed upon any variant from the expected classes due to contamination; for the variant, if arising from the cross, would give some offspring in the F_2 generation with some of the recessive characters. However, extreme care was taken to avoid contamination and at no time was there reason to suspect it in any of the cultures.

The wild stocks used were from Arkansas, California, Massachusetts, Illinois, Minnesota, Ohio, Wyoming, Porto Rico, Cuba, Australia and France. The totals of the F_2 generations are as follows:

	Ch. Cl. Ver.	Bl. Pk. B.	Bl. P. Vg. Arc. Sp.
Arkansas	1,162	307	198
California	859	715	332
Illinois	211		287
Massachusetts	1,078	681	1,013
Minnesota	771	274	
Ohio	506	1,612	370
Wyoming	925	150	
Porto Rico	151	207	
Cuba			819
Australia	469	401	548
France	814	951	826
Total	6,946	5,298	4,393

This gives a grand total of 16,637 flies. It should be noted that these flies were examined with the greatest care under a binocular microscope. Each fly was turned over separately and every part carefully examined.

From the cherry club vermilion crosses the following abnormal forms were found; three gynandromorphs; twenty-four flies with more or less beaded wings; two flies with three cross veins on the wings; one truncate; and two flies with abnormal abdomen.

The abnormal forms from the crosses with black purple vestigial arc speck were, sixty-three with more or less beaded wings; one truncate; one abnormal abdomen; one fly with five legs; and four flies with a projection from the posterior cross vein toward the base of the wing.

From the black pink bent crosses were found two beaded; one abnormal abdomen; three truncate; and one called furrowed because of the furrows in the eyes due to the foreshortening of the head.

This gives a total of 109 abnormal forms or one abnormal in

every 152 flies. But 89 of these abnormals were flies with beaded wings. This character is very variable; some of the flies had only a few bristles missing from the margin of the wings, while others had both the outer and inner margins of the wings serrated. The character has been recurring in the stock so frequently that it can scarcely be ascribed to outcrossing. Many of these flies were mated, but they either did not leave offspring, or the character did not reappear in the F_2 generation.

The three gynandromorphs are not to be considered as mutants. The data here show that gynandromorphs occur once in about five thousand five hundred times.

Flies with truncate wings are of occasional occurrence in the laboratory stock, as are also those with abnormal abdomen; hence, flies with these characters are not to be considered as due necessarily to the outcrossing. The truncate would not breed and the abnormal abdomen character did not reappear in the F_2 generation. If a character does not reappear in the F_2 generation it is considered to be of somatic and not of germinal origin, unless an environmental condition is necessary for the expression of the changed character.

The abnormality of the fly with five legs may have been the result of accident, for the character did not reappear in the F_2 generation.

Three characters were found to be inherited; the one called "furrowed," which arose from the cross of black pink bent with wild stock from Massachusetts; the one with a projection from the posterior cross vein toward the base of the wing, called "tau," which arose from the cross of black purple vestigial arc speck with wild stock from Illinois; but since this stock had just been received from Illinois, and since the character appeared in four of the flies, it is suspected that the character was recessive in the wild stock and not due solely to the cross. Also from cherry club vermilion crossed to stock from Arkansas arose two males with three cross veins on the wings and a disturbance of the ommatidia of the eye. This character is called "warty."

Pure stocks of flies with these characters have been bred for many generations and each continues to breed true. "Warty" has many other characters than the modification of the eyes, *e. g.*, beaded wing, spread wing, from two to five cross veins on the wings, abnormal abdomen and disarranged hairs on the thorax. The females are sterile and the race is maintained

by crossing the males to their heterozygous sisters. The character is not sex linked; it decreases the viability of the flies, but more than this can not be said at present. Work is being continued on this character and on flies with the character "tau."

"Furrowed" is characterized by having the head foreshortened, which causes indentations or furrows in the eyes; also the spines on the scutellum are stumpy. The last character is of importance in determining some of the flies, as a female will sometimes occur without any disturbance of the eyes.

This character arose in a male which was crossed to a wild female. The F_2 generation gave normal females and half the males were normal and half were furrowed. This established the fact that the character followed the distribution of the sex chromosome. The position of the gene in the chromosome was next determined according to the theory that the genes in any chromosome are arranged in a linear series.[1] Crosses were made with eosin miniature, sable forked, and with vermilion barred. Because of the low fertility of the furrowed females the cross was always made with the furrowed males. Consequently, the males alone are considered in the counts given below.

EOSIN MINIATURE ♀ BY FURROWED ♂

F_2 males	Normal	3	Furrowed	67
	Eosin miniature furrowed	1	Eosin miniature	75
	Eosin long	3	Miniature	31
	Miniature furrowed	0	Eosin long furrowed	28

In the first column are the cross-over classes between miniature and furrowed and the per cent. of these to the whole number is 3.4. Then the gene which determines the character "furrowed" is supposed to lie 3.4 points beyond miniature, or at 39.6.

SABLE FORKED ♀ BY FURROWED ♂

F_2 males	Furrowed sable forked	1	Sable forked	61
	Normal	8	Furrowed	105
	Forked	3	Furrowed forked	15
	Furrowed sable	0	Sable	16

In the first column are the cross-over classes between furrowed and sable and these are 5.7 per cent. of the entire number. Then furrowed lies at a point 5.7 to the left of sable, or at 37.3.

[1] Sturtevant, *Jour. Ex. Zool.*, '13.

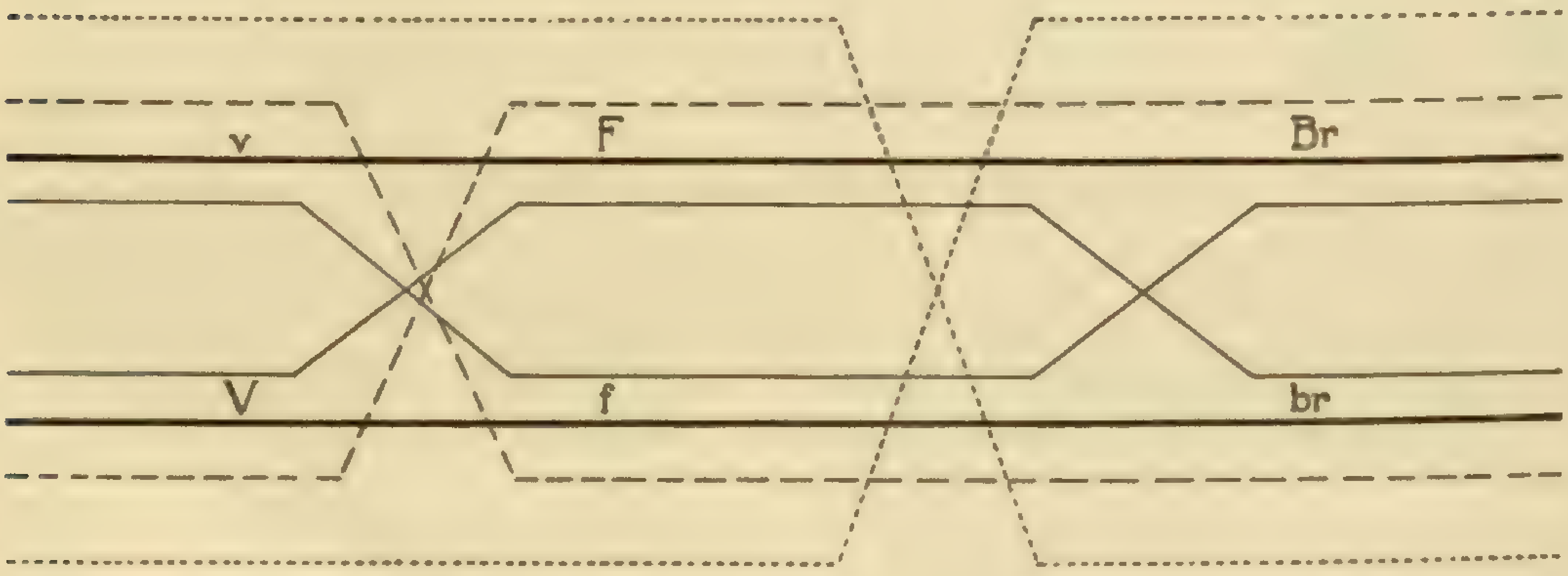
VERMILION BARRED ♀ BY FURROWED ♂

F_2 males..	Bar	6	Vermilion bar	86
	Vermilion furrowed	3	Furrowed	102
	Normal	0	Furrowed bar	15
	Vermilion furrowed bar	0	Vermilion	28

The cross-over classes between vermilion and furrowed are the bar and vermilion furrowed classes of which there are nine, which is 3.75 per cent. of the entire number. Vermilion is at 33, hence the gene for furrowed lies at 36.75.

The cross-over classes between furrowed and bar are the furrowed bar and the vermilion classes of which there are 43 which is 18 per cent. Then furrowed lies 18 points to the left of bar or at 39.

The discrepancy in these results is due to the low viability of the furrowed flies, yet the results agree fairly well, varying from 36.75 to 39, giving an average of 38.1, which is considered as the relative position of the gene for furrowed in the sex chromosome.



The accompanying diagram will aid in understanding the cross-over classes. The heavy straight lines represent the paired sex chromosomes which a heterozygous female has received from her parents. The upper one, which carries vermilion bar, was received from the female parent and the lower, carrying furrowed, was received from the male parent. Each of the sons of this heterozygous female receives one of these chromosomes which determines what it shall be with reference to these special characters. In about 75 per cent. of the cases the sons receive these chromosomes without any interchange of substance between the two as is shown by the two straight lines which represent the non-cross-over classes. When there is an interchange

of material between the two chromosomes as indicated by the crossed lines, then males arise with a different arrangement of the characters from that which had appeared in the grandparents.

In the diagram v, f and Br stand for vermilion eye, furrowed eye, and bar eye, respectively; while V, F and br stand for the normal allelomorphs of these characters, *i. e*,. red eye, not furrowed and not bar. Reading from the left the top dotted line includes v, F and br, but since F and br are normal the flies will differ from normal forms in the one character alone, viz., vermilion. The dotted line below includes V, f and Br, hence the males receiving this chromosome are furrowed bar. Referring to the table showing the cross between a vermilion bar female with a furrowed male we see that there were 28 vermilion and 15 furrowed bar flies. Reading from the left again and omitting the normal allelomorphs, the upper dash line includes vermilion and furrowed and the lower dash line includes bar alone. The table shows that there were only three vermilion furrowed and six bar males, hence the interchange of material between vermilion and furrowed took place less frequently than it did between furrowed and bar. Since the per cent. of crossing over between any two genes is taken as the index of the relative distance between those genes, then furrowed lies much closer to vermilion than it does to bar.

The fine lines represent double crossing over, of which no representatives were found in this cross.

Summary and Conclusions

Crosses were made with mutant stocks of *Drosophila* with wild stock from many localities in the United States, from the West Indies, France and Australia in order to discover, if possible, if hybridization is an essential factor in the formation of mutant races. From 16,637 flies of the F_2 generation seven flies arose which varied from the normal type and which bred true. If we discard the four with the character "tau" for reasons given above, then the result is narrowed to three flies with two characters. This gives one mutant to every 5,545 flies. Therefore, a mutation has occurred so seldom that we can scarcely attribute hybridization as its cause. It is highly probable that if the same number of wild flies had been reared under

favorable conditions for the survival of any new forms that appeared just as many mutations would have been found as in the above experiment. In the light of these results we can attribute the origin of mutations only to chance, since hybridization as a causal agent does not occupy a privileged position relative to the effect.

F. N. DUNCAN

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LINKAGE AND SEMI-STERILITY

THE Florida velvet bean (*Stizolobium deeringianum*) has normal pollen and embryo-sacs; it flowers (when sown in May) early in September; and has pigmented (mottled) seed-coats. The Yokohama bean (*Stizolobium hassjoo*) has also normal pollen and embryo-sacs; it flowers in July; and has its seed-coats unpigmented. The first-generation hybrids of Florida by Yokohama had half their pollen and embryo-sacs aborted (1, 2); flowered at the end of August; and had more or less pigmented seed-coats. In the second generation, half of the plants had normal pollen and embryo-sacs, and half showed semi-sterility (1, 2). These plants flowered from July to September, the majority being late. About three-quarters had pigmented seed-coats; and one-quarter, colorless seed-coats.

Most of the semi-sterile plants, and also most of the plants with pigmented seed-coats, were late in flowering. The semi-sterile plants, however, were not later than the fertile, in the second generation of the *Florida by China* cross. Hence there is no necessary connection between semi-sterility and lateness. A random sample of five second-generation plants of the Florida by Yokohama cross gave one family with pigmented seed-coats, one family with colorless seed-coats, and three families segregating into pigmented and colorless in about the ratio 3 : 1. Hence the pigmentation of the seed-coat is not a mere physiological consequence of lateness, but is determined by a definite factor. If *K* is the factor from the Florida concerned with semi-sterility; *P*, a factor concerned with pigmentation of seed-coat; and *H*, the main factor for lateness; then *K* and *H* are strongly coupled in the gametes of the first-generation plants, as are also *P* and *H*. *K* and *P* show secondary coupling.

The data follow.

SEMI-STERILITY AND LATENESS

Second generation of Florida by Yokohama
Seeds sown early in May
(Classes are approximately fortnights)

	1	2	3	4	5	6	Totals
Fertile plants	14	29	19	7	5	7	81
Semi-sterile plants	6	10	11	29	10	9	75

The average of the semi-sterile is about a fortnight later than that of the fertile. If we divide the plants into those flowering before and after August 11, we have:

	First Month	Second and Third Months
Fertile	43	38
Semi-sterile	16	59

A calculation, based on the hypothesis used for semi-sterility (1), shows that the crossing-over (3) between K and H is probably less than 17 per cent.

SECOND GENERATION OF FLORIDA BY YOKOHAMA

Seeds sown early in June

	2	3	4	5	6	Totals
Fertile	7	24	33	5	8	77
Semi-sterile	4	17	28	14	25	88

The fertile plants are earlier than the semi-sterile; though the average difference is less than in the early planting, because, as usual, the first-early plants are more affected by late planting than are the later plants.

PIGMENTATION OF SEED-COAT, AND LATENESS

Second Generation of Florida by Yokohama
Early sowing

	1	2	3	4	5	6	Totals
Unpigmented seed-coats	16	18	6	0	0	1	41
Pigmented seed-coats	4	22	25	35	14	12	112

Thus most plants with unpigmented seed-coats are early. A calculation again shows that the amount of crossing-over is probably under 23 per cent.

SECOND GENERATION OF FLORIDA BY YOKOHAMA

Late sowing

	2	3	4	5	6	Totals
Unpigmented seed-coats	8	20	12	1	2	43
Pigmented	3	21	46	17	31	118

This confirms the results from the early sowing.

PIGMENTATION OF SEED-COAT AND SEMI-STERILITY

The coupling between K and P is given from the following:

SECOND GENERATION OF FLORIDA BY YOKOHAMA

	Unpigmented	Pigmented	Totals
Fertile	45	110	155
Semi-sterile	39	120	159

The excess of pigmented semi-sterile and of unpigmented fertile testifies to a slight coupling, and calculation shows that there is probably about 35 per cent. of crossing-over.

According to the hypothesis (1), fertile second-generation plants should be mainly homozygous for H (or h) and P (or p); while semi-sterile plants should be mainly heterozygous for these factors. This is being further tested.

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EARLY PORTRAYALS OF THE OPOSSUM

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THE quaint animal figures found in olden time works on natural history are interesting not only as bearing upon the contemporary state of zoological science and the art of book-making, but also because many of the illustrations belong to a regular sequence or lineage which can be traced back, like the textual descriptions, to primitive sources. To a certain extent this has already been done, or at least indicated, in the work by John Ashton, entitled "Curious Creatures of Zoology."

A subject deserving of the attention of naturalists but which appears to have been neglected, is an historical and systematic investigation of animal figures introduced in early American cartography. Thanks to the magnificent facsimile reproductions of sixteenth century maps which have been published during recent years in this country and abroad, abundant materials for this purpose are now easily accessible. As for the "relaciones" of early voyagers and travelers in the western world, very few of these have been published with scientific commentaries, and among the really important seventeenth century writers on Central and South American natural history, only the works of Hernandez (1628) and Marcgrav[1] (1648) have been systematically annotated. The first letter

[1] See the commentaries on these authors by Lichtenstein and Martius, 1827 and 1853, in the publications of the Berlin and Bavarian Academies of Science.

written from the newly discovered world, by Dr. Chanca, companion of Columbus, was not adequately edited and annotated until after four centuries had passed. Vespucci's letters also are deserving of mention in this connection.[2]

In view of the fact that several communications have appeared in *Nature* during the past year concerning the first mention of the opossum in literature, it may not be inopportune to trace the pedigree of some of the early illustrations of this animal, both in maps and in printed works. At the same time a few of the older printed descriptions of American marsupials may be noticed. And we will observe first of all that the earliest reference to the common American opossum is found in the famous collection of voyages published in 1504 by Angelo Trivigiano, under the caption of "Libretto de Tutta la Navigatione de Re de Spagna, de le Isole et Terreni Novamente Trovati." In Chapter XXX of that work it is mentioned that a live specimen, taken by the Pinzons in Brazil in 1500, was exhibited in Granada.

In Decas II of Peter Martyr's "De Nove Orbe," published in 1511, occurs the first published description of the American tapir; and immediately following this the opossum is referred to in these words:

> There is also an animal which lives in the trees, feeds upon fruits, and carries its young in a pouch in the belly; no writer as far as I know has seen it, but I have already sufficiently described it in the Decade which has already reached Your Holiness before your elevation, as it was then stolen from me to be printed.

In 1547 and 1548, and again from 1549 to 1555, Hans Stade of Homburg, Hesse, passed some time in Brazil, and wrote or dictated an account of his strange adventures, which was published at Marburg in 1557. Under the caption of "Servoy," Chapter XXXII, we read:

[2] See Fernandez de Ybarra in *Journ. Amer. Med. Assoc.* for September, 1906, and in Misc. Coll. Smithson. Inst. for the same year. Vespucci's first letter (1497) was republished in facsimile by Varnhagen in 1893, having for frontispiece a design by Stradanus dating from about 1580, in which various South American animals are well represented. Mention occurs in this letter of the iguana, puma and ocelot from the coast of Tampico.

There is also a kind of game, called *servoy,* which is as large as a cat, and has a tail like a cat; its fur is gray, and sometimes grayish black. And when it breeds, it bears five or six young. It has a slit in the belly about half a span in length. Within the slit there is yet

FIG. 1. The "Dattu" (tatou or armadillo) and "Servoy" (opossum); after Hans Stade, 1557.

another skin; for its belly is not open, and within this slit are the teats. Wherever it goes, it carries its young in the pocket between the two skins. I have often helped to catch them and have taken the young ones from out of the slit.

In the original edition of the work just quoted woodcuts are given of both the opossum and armadillo (*Dasypus novemcinctus* Linn.) and these are reproduced in the present article (Fig. 1) from a copy belonging to the New York Public Library. The armadillo is thus described in Stade's "Wahrhaftig Historia":

There is another sort of animal found in this country which the savages call *dattu;* it stands about six inches high and is nine inches long; its body is covered all over, except underneath, with a kind of armor. This covering is horn-like, and the plates overlap one another like those of chain armor. This animal has a very long snout, and is usually found on rocks. It feeds on ants. Its flesh is sweet and I have often eaten of it.

Two works published at about the same time as the narrative of Stade also contain mention of the opossum, the name of "Simivulpa" or Fox-ape and "Su" being

FIG. 2. The "Simivulpa" (*Didelphis*) ; after Sebastian Münster, 1558.

applied to the creature. In the Italian edition (1558) of Sebastian Münster's "Cosmographia" occurs this passage, accompanied by an illustration which we have reproduced in Fig. 2:

Trovasi in quel luogo [Brazil] un animal prodigioso, le cui parti davanti si rassomigliano a volpe & quella di dietro à Simia mai suoi piedi sono como di huomo, ha le orecchi di civetta, & sotto le ventre como una borsa, nella quale tien nascosti suoi figliuoli, finche crescono di sorte che possino caminare securamente da lor stesi, & procurarsi il cibo senza tutela della madre, ne mai escono di quella borsa se non quando lattano. Quest' animale mostruosa con tre suoi figliuoli fu portato in Sibilía & indi in Granatá."—p. 1187.

Münster's illustration of the "Simivulpa" is evidently derived from figures of the opossum appearing in several editions of Ptolemy's "Geography" from 1522 onward,

FIG. 3. Earliest known figure of the opossum: from the Waldseemüller world-map of 1516.

and other early maps of South America, all traceable in the first instance to Waldseemüller's world-map of 1516, where the same representation occurs (Fig. 3). It is there accompanied by essentially the same legend as one finds in the "*Tabula Terre nove*" of the 1522 Ptolemy, and in later maps and atlases,[3] such as Cornelius de Jode's (1585), and van Linschoten's (1598).

[3] Modern reproductions of South American maps showing these figures may be seen in Winsor's "Narrative and Critical History of America," and in the magnificent collection published by the Brazilian government under the direction of Baron de Rio Branca. The representation of a Brazilian landscape in the Cantino map of 1500, shown in our Fig. 4, is from a photograph of Harrisse's colored reproduction.

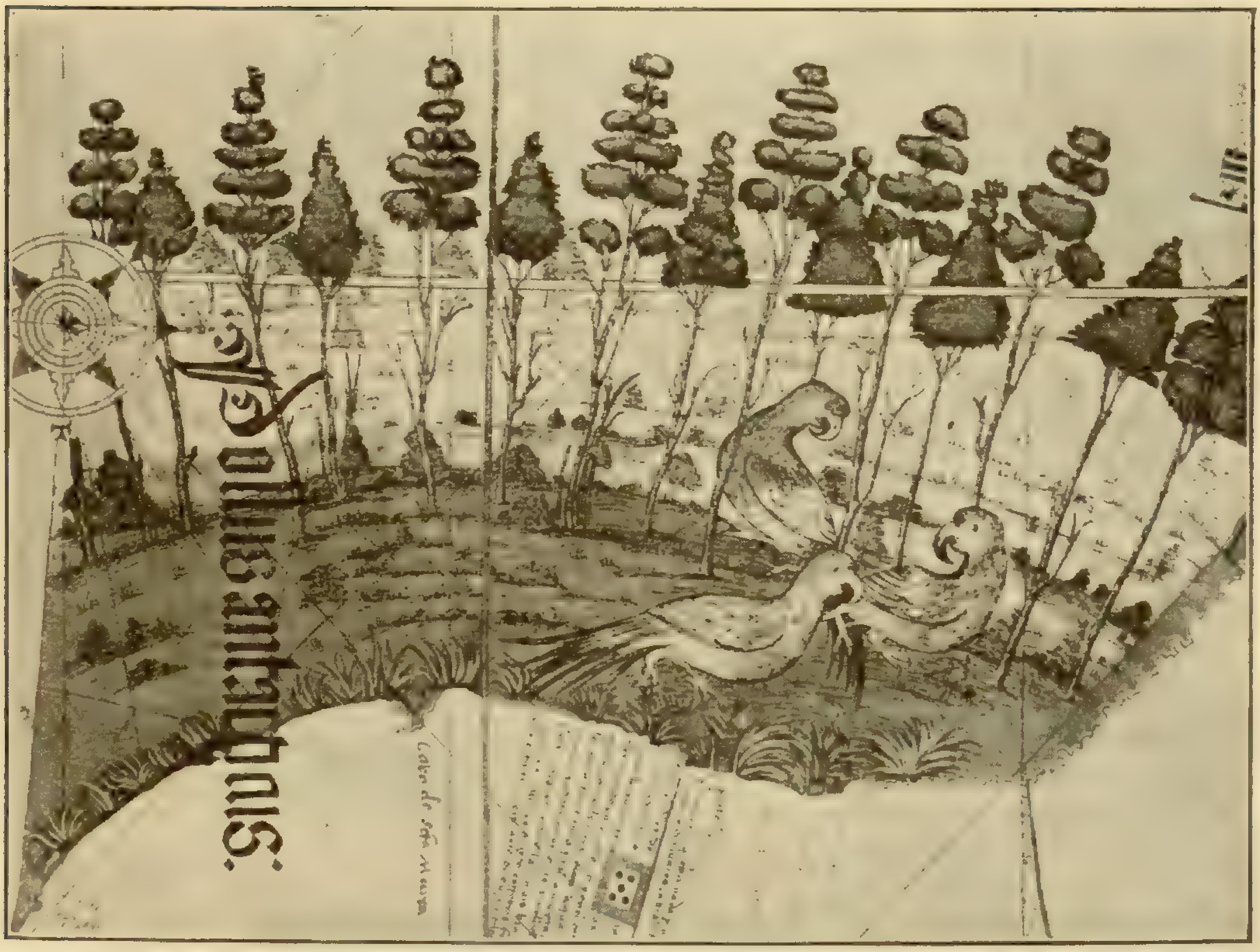

Fig. 4. One of the earliest representations of an American landscape; from the Cantino map of 1500.

André Thevet, who sojourned for a short time in Brazil, published his "Singularitez de la France Antarctique" in 1558. His description of the "Su," in reality the opossum, is paraphrased by Conrad Gesner, Edward Topsell, J. E. Nieremberg and John Jonston under that caption, and his grotesque caricature of the beast is reproduced by these authors. It is also introduced in sixteenth century cartography of the two Americas. Blaeu, in his world-map of 1605, places the "Su" and its descriptive legend in the region of Nova Francia;[4] and in the La Plata region of the same map occurs still another figure of the opossum, based upon the century-old drawing which appears in the Waldseemüller world-map. Our Fig. 5 is taken from Thevet, and Fig. 6 from Nieremberg, whose "Historia Naturæ" was published in 1635.

In Wolfe's English edition of van Linschoten's "Voyages," figures of the sloth and "Simivulpa" are intro-

[4] See the new facsimile edition (1914) published by Dr. E. L. Stevenson under the auspices of the Hispanic Society of America.

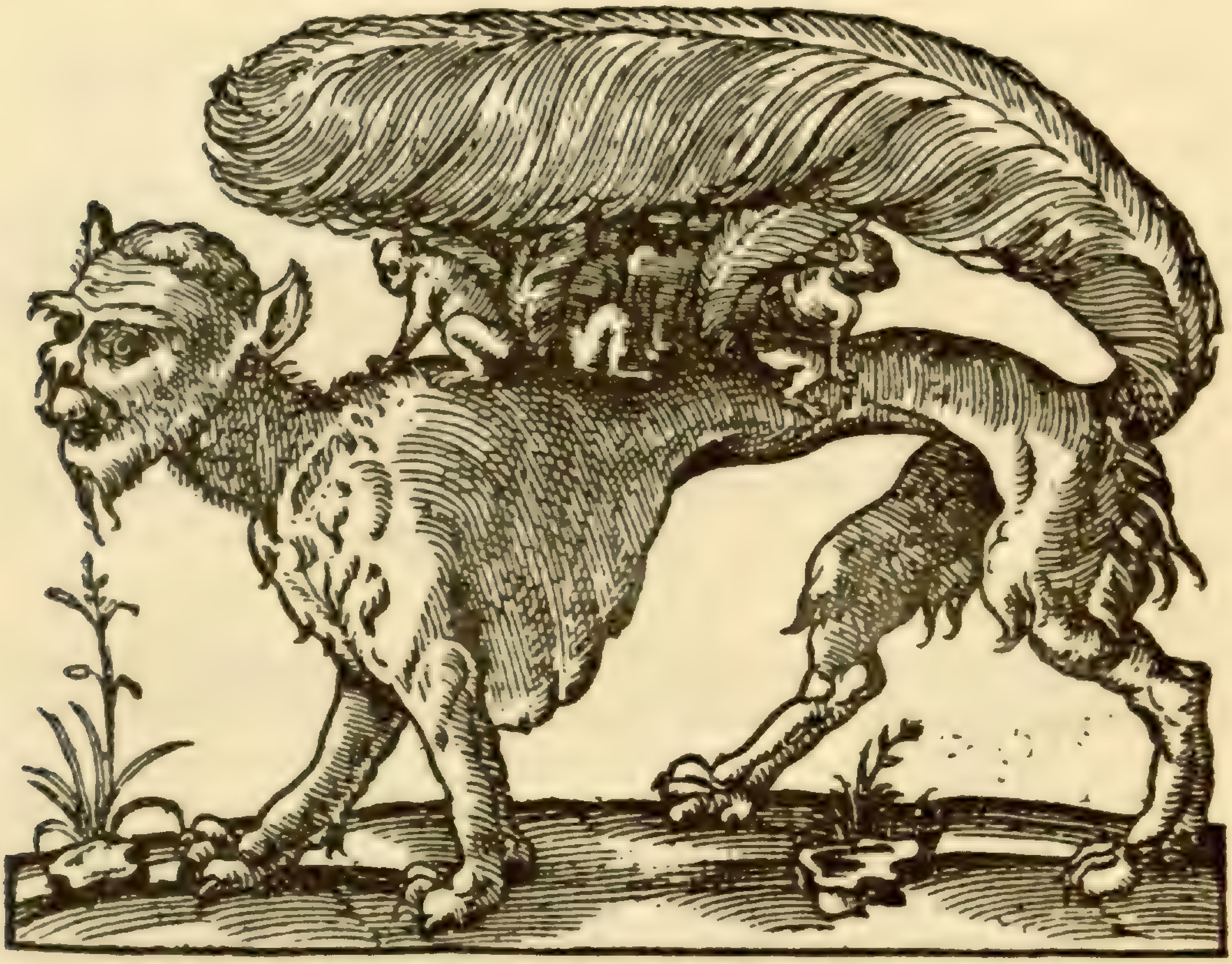

FIG. 5. The "Su" (common opossum); after André Thevet, 1555.

FIG. 6. The "Flaquatzin" (wooly opossum); from Topsell, after Nieremberg, 1635.

duced in the Brazilian and Argentine region of the map of the South American continent, and at page 232 of this work occurs the following description of one of these beasts:

There is likewise another wonderful and strange beast of Gesnerus called a Foxe ape, on the belly whereof Nature hath formed an other belly, wherein when it goeth into any place, it hideth her young ones, and so beareth them about with her. This beast hath a body and members like a foxe, feete like mens hands, or like sea cattes feete, eares like a batte. It is never seene that this beast letteth her young ones come forth but when they sucke, or ease themselves, but are alwayes therein, until they can gette their own meate.

Passing now to the seventeenth century writers, we find this account of *Didelphis* in Raphe Hamor's "True Discourse of the Present Estate of Virginia" (London, 1615):

For true it is, that the Land is stored with plenty and variety of wild beastes, Lions, Bears, Deere of all sorts. . . . Beavers, Otters, Foxes, Racounes, almost as big as a Fox, as good meat as a lamb, Hares, wild Cats, Muske rats, Squirrels flying, and other of three or foure sorts, Apossumes, of the bignesse and likenesse of a Pigge, of a moneth ould, a beast of as strange as incredible nature; she hath commonly seauen young ones, sometimes more and sometimes lesse, which she taketh vp into her belly, and putteth forth againe without hurt to her selfe or them.

Of each of these beasts, the Lion excepted, my selfe have many times eaten, and can testifie that they are not only tastefull, but also wholesome and nourishing foode.

FIG. 7. The opossum and young; after César de Rochefort, 1658.

About the same time Captain John Smith wrote the following brief characterization of the opossum, in his "Description of Virginia" (1612):

An opossum hath a head like a Swine, and a taile like a Rat, and is of the bignesse of a Cat. Under her belly she hath a bag, wherein she lodgeth, carrieth, and suckleth her young.

After Nieremberg, a Jesuit professor at Madrid, whose work on natural history (1635) is chiefly a compilation, we come to George Marcgrav and Wilhelm Piso; and

their contributions on Brazilian natural history, published in 1648, are recognized as highly meritorious.

Ulysses Aldrovandi's large posthumous folio on Quadrupeds (1637, p. 103) also contains a figure of the opossum (otherwise interpreted, however) which is clearly traceable to the early cartographic designs. But it is unnecessary to pursue the subject further, except to state that Fig. 7 is copied after Charles César de Rochefort's engraving of an opossum ("Histoire des Îles Antilles," 1658), and Fig. 8 shows the same animal, acording to Eduard Seler's interpretation, as depicted in one of the Maya Codices (Nuttall, 71).

FIG. 8. Maya representation of the opossum (?) From Eduard Seler, after Nuttall Codex, 71.

Among other mammalian figures in pre-Columbian Maya and Mexican colored drawings[5] that have been preserved are several that represent a spotted dog, probably one of the varieties of "Alcos" mentioned by Hernandez. The occurrence of an indigenous spotted dog in Central America is of interest in view of the fact that a similar race is depicted in ancient Egyptian, Assyrian and Pelasgian animal effigies and paintings, some of the figures dating as far back as about 3000 B.C.

The oldest known representations of the hunting dog of the ancient Egyptians, together with a number of large African mammals, are inscribed in a palette discovered a few years ago at Hierakonpolis.

[5] See Edward Seler, "Die Tierbilder der mexicanischen und Maya-Handschriften," *Zeitschr. f. Ethnol.*, Jhrg. 41, 1909. A. M. Tozzer and G. M. Allen, "Animal Figures in the Maya Codices," papers of Peabody Museum Arch. Ethnol., Vol. 4, No. 3, 1910. References to the literature on ancient Egyptian and Assyrian animal effigies will be found in *Amer. Journ. Philol.*, Vol. XXX, 1909, pp. 322–331. The early history of the rhinoceros is traced by B. Laufer in Publication 179 of the Field Museum, and medieval ideas of the elephant are portrayed by E. D. Cuming in a recent number of *Field* (April 3, 1915).

Concerning the several varieties of ancient Inca or Ancon dog that are known from well-preserved Peruvian mummies, Nehring[6] is of the opinion that their remote ancestry is traceable to the North American wolf (*Lupus occidentalis* var. *mexicanus* and *rufus*). The great antiquity of domesticated dogs in South America is indicated also by a canine skull which R. Lydekker has described from the superficial deposits of Buenos Aires. This dog, according to Dr. Lydekker,[7] "though apparently contemporaneous with many of the wonderful extinct mammals of the Pampas, yet shows unmistakable signs of affinity with domesticated breeds, although the precise relationship has not been established."

Reference having already been made to animal figures in early American cartography, we may call attention in closing this sketch to a memoir by Aníbal Cardoso in the *Anales* of the Buenos Aires Museum for 1912 (Vol. XV), on the origin of Argentine horses.[8] The writer endeavors to show from historical evidence that large numbers of horses existed in the interior of the country prior to the Spanish Conquest, and a figure of one of these animals drawn by Sebastian Cabot in his world-map of 1544 is interpreted as indicating that wild herds were seen by that navigator in 1531. A portion of Cabot's map is reproduced in Señor Cardoso's memoir (p. 379), and also in one by J. T. Medina on the voyage of Sebastian Cabot.

Nevertheless the conclusion appears unavoidable that, had the horse actually persisted in the western hemisphere down to the time of the advent of Europeans, some traces of it would certainly appear in the culture of the primitive inhabitants.

[6] *Sitzungsber. ges Naturf. Freunde*, Berlin, 1884.

[7] R. Lydekker, "Mostly Mammals," London, 1903, p. 204.

[8] "Antigüedad del Caballo en el Plata." On the horse in post-conquistorial times in North America see Clark Wissler, "The Influence of the Horse in the Development of Plains Culture," in *Amer. Anthropol.*, Vol. XVI, 1914.

SEVENTEEN YEARS SELECTION OF A CHARACTER SHOWING SEX-LINKED MENDELIAN INHERITANCE[1]

RAYMOND PEARL

I

In 1898 there was begun at the Maine Agricultural Experiment Station an experiment in breeding Barred Plymouth Rock fowls, having for its purpose the improvement by selection of the character winter egg production. This investigation has continued to the present time. A résumé of the results to date, considered with reference to their bearing upon the general biological problem of selection, may be of some interest.

The experiment has fallen into three divisions or periods: viz., (1) the period from 1898 to 1907, (2) the period from 1908 to 1912, and finally (3) the period from 1912 to date. Detailed reports on the methods of breeding in operation have been published elsewhere.[2] For purposes of clear orientation in the present discussion it will be well here briefly to review the facts as to the methods of breeding used in each of the periods. With these facts definitely in mind we may then proceed to an examination of the results.

1. *The Period from 1898 to 1907.*—During this period the breeding followed the plan outlined at the beginning by Woods and Gowell. Essentially it consisted of the following elements.

1. Trap-nest record of the performance of each individual female.
2. Selection as breeders of all females which laid more than a definite number of eggs (150) in the first laying year.

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 87.

[2] Cf. particularly Woods, C. D., and Gowell, G. M., U. S. Dept. Agr. Bur. Anim. Ind. Bulletin 90, 1906, pp. 42; Pearl, R., and Surface, F. M., *Ibid.* Bulletin 110, Part I, 1909, pp. 80; Pearl, Me. Agr. Expt. Stat. Ann. Rept., 1911, pp. 113–176; and Pearl, *Jour. Exp. Zool.*, Vol. 13, 1912, pp. 153–268.

3. Selection as breeders of males whose dams had laid more than another definite number of eggs (200).
4. The indiscriminate mass breeding, *without* individual pedigrees, of all individuals selected as described under 2 and 3, and, in consequence,
5. *No test of the progeny* of particular matings with respect to their laying ability.

This may be designated as the *period of mass selection.*

The following statement regarding the methods used in this period was made by Woods and Gowell (*loc. cit.*, p. 8):

The plans followed in this breeding work are based upon everyday, practical common sense, and are the same as would be used in building up a high-producing strain of dairy animals. Individual records of performance are kept. The large producers are mated with sons of large producers in the hope of obtaining a race of improved layers. In the first year's work three birds laid over 200 eggs each, and this fact led to the adoption of that number of eggs as the minimum performance for a "registered" bird. Other than this there was no reason for selecting 200 as the number of eggs necessary to entitle a bird to registration. Any other number, as 190 or 210, might have been taken with equal propriety, just as horsemen might have selected some other time than 2.30 by which to determine a standard horse.

2. *The Period from 1908 to 1912.*—For reasons which have been fully set forth elsewhere[3] it was decided not to continue the breeding along the same plan after 1907. The new plant, put into operation first in the breeding season of 1908, was calculated primarily to furnish definite information regarding the mode of inheritance of the character winter egg production. It involved essentially the following items:

1. Trap-nest record of the performance of each individual female.
2. The selection of both males and females was made on a *double* basis, including in addition to the individual's own performance as in the earlier plan, also the idea of progeny performance. In practice this worked out *for hens* in the following way: Plans were made to see whether there could

[3] Pearl and Surface, *loc. cit.*

be formed by selection and propagated three distinct strains of winter egg producers, namely, high, mediocre and low. This involved, *on the individual performance side,* the separate selection in the first years of *three* classes of females as breeders: (*a*) good winter producers, with records before March 1 of above 30 eggs; (*b*) mediocre winter producers, with records below 30 eggs; and (*c*) poor winter producers, which laid no eggs before March 1. The division at 30 eggs was, after the first year, merely a nominal one in the selection of *high* producers. Actually only birds were used in the *a* class whose records materially exceeded 30 eggs, running up to over 100 eggs in some cases.

The *progeny performance* idea was carried out in two ways in the breeding. In the first place, no female was selected for the *high* winter production breeding pens, for example, unless, in addition to her own high winter record, all her sisters and her dam were high producers. In the second place, of all females fulfilling the above qualification only those were bred a second time whose progeny from the first year's mating had proven to be all high producers. Similar types of selection were followed by the mediocre and low lines, except that segregating families were put in the mediocre class.

3. The selection of males was along essentially the same lines, with only such difference as is involved in the fact that the male makes no performance record himself. Males were put into the breeding pen the first time on the basis of the records of their dams, on the one hand, and of their sisters, on the other hand. Those whose progeny proved that they were transmitting the character to which selection was being made were used a second or even third time as breeders.

4. Complete individual pedigrees, whereby each off-

spring individual's parentage, both male and female, was known.

5. The records of production of the progeny of each mating separately recorded and studied as a unit.

It will be noted that there are but two essential differences between the plan in this period and that followed in the earlier one. These are: first and most important, that in this second period the principle of *progeny testing* was introduced into the scheme of breeding. The second difference was that selection was carried on for low production as well as for high, which had not been previously done. A third difference is apparently found in the fact that in this second period of selection the winter record rather than the yearly record is made the basis of selection. This is in no way an essential difference. The reasons for adopting the winter period have been set forth in detail elsewhere[4] and need not be repeated. It suffices to say here that essentially the same results and conclusions will be reached if one uses winter production or annual production.

As a result of the studies made in this period on the plan of breeding outlined the mode of inheritance of the character winter production was definitely determined, and has been confirmed by subsequent work.[5] The character was shown to be Mendelian in its genetic behavior, depending upon two factors, one of which is sex-linked.

3. *The Period from 1912 to Date.*—The only difference in the mode of breeding the stock of Barred Plymouth Rocks in this period, as compared with the preceding one, is found in the fact that during this last period *all selections for low and mediocre production have been dropped.* The breeding for high production alone continues, with only such differences in the details of manipulation of the breeding stock as would naturally follow a definite knowledge of the mode of inheritance of the character,

[4] Pearl, 1912, *loc. cit.*, and Me. Agr. Exp. Stat. Ann. Rept., 1914, pp. 217–236. Cf. also Wilson and Murphy, *Jour. Dept. Agr. Ireland*, Vol. XIV, No. 2.

[5] Pearl, 1912, *loc. cit.*, also Amer. Nat., Vol. XLIX, 1915, pp. 306–317, and Curtis and Pearl, *Jour. Exp. Zoology*, Vol. 19, 1915, pp. 45–59.

and of the gametic constitution of particular individuals with reference to that character. As a matter of fact, all low-producing lines were dropped at the end of the laying year 1911–12. Certain of the mediocre lines were continued a year longer. In the laying flock of 1913–14 there were no birds which had been bred for anything other than high production, so far as the breeder's deliberate intention went.

II

The results of this seventeen year selection period are set forth in Table I.

TABLE I

MEAN WINTER PRODUCTION PER BIRD OF THE BARRED PLYMOUTH ROCK FLOCKS FROM 1899 TO 1915

Laying Year	Mean Winter Production of All Birds	No. of Birds Making Winter Records	Mean Winter Production of All Birds Selected for *High* Production	Mean Winter Production of All Birds Selected for *Low* Production
1899–1900	41.03 eggs	70	—	—
1900–1901	37.88 "	85	—	—
1901–1902	45.23 "	48	—	—
1902–1903	26.01 "	147	—	—
1903–1904	26.55 "	254	—	—
1904–1905	35.04 "	515	—	—
1905–1906	40.65 "	635	—	—
1906–1907	22.44 "	653	—	—
1907–1908	19.93 "	780	—	—
1908–1909	26.69 "	359	54.16	22.06
1909–1910	31.76 "	247	47.57	25.05
1910–1911	30.49 "	264	50.58	17.00
1911–1912	35.93 "	232	57.42	16.43
1912–1913	43.01 "	182	52.61	—
1913–1914	52.20 "	192	52.20	—
1914–1915	45.89 "	179	45.89	—
Totals and means	35.05 "	4,842	51.49	20.14

The data of this table are shown graphically in Fig. 1.

From the table and diagrams the following results appear:

1. The number of individuals involved in this experiment, on each one of which exact trap-nest records have been kept, is large, amounting nearly to five thousand. This number is large enough to lead to conclusions which are trustworthy. It will be shown presently that wherever it has been possible to compare the results on egg produc-

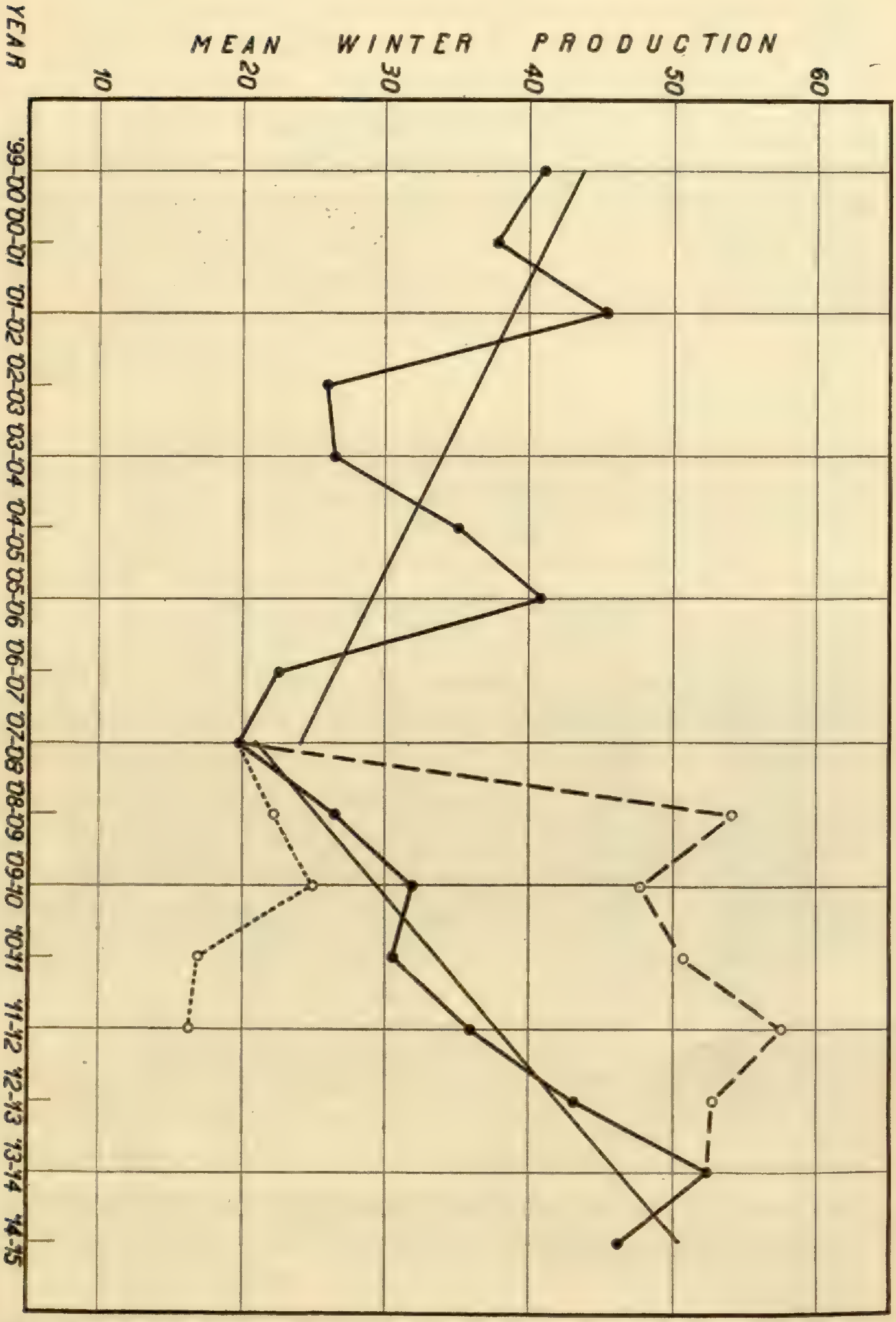

FIG. 1. Graph showing the course of mean winter egg production between the years 1899 and 1915. The solid lines and circles give the total flock means. The two straight lines, fitted by the method of least squares to the observed flock means, have the equations, $y = 43.655 - 2.181x$, and $y = 17.070 + 4.148x$. The open circles and broken (dash) line give the means of the lines selected for high winter production between the years 1908 and 1915. The dotted line and open circles give the mean winter production of the lines selected for low production between the years 1908 and 1912.

tion obtained in this experiment with independently determined norms the general trustworthiness and normality of the present data have been demonstrated.

2. From the beginning of the experiment through the laying year 1907–08 the general trend of mean production was downward, with minor fluctuations from year to year. In other words during the period in which the system of breeding was mass selection for high production without progeny test there was no change of the mean in the direction of the selection. The fluctuations in mean production during this period were, in the main, due probably to two sets of causes: (*a*) environmental differences in different years acting at one point or another in the life history of the birds; (*b*) random fluctuations in the genetic constitution of the male birds used as breeders in successive years, brought about because of the ignorance of the breeder, in the absence of any individual progeny testing plan, of the ability of any particular male to transmit high fecundity to his daughters. The first of these factors needs no special discussion and is relatively of minor importance. The second will be dealt with in detail farther on in the paper.

3. Since the laying year 1907–08 there has been a steady increase in mean winter production for the whole flock, except for the years 1910–11 and 1914–15. In the former year the decline in the mean is slight, and is probably due to unfavorable environmental influences. In 1914–15 the decline is certainly due to such causes. In the hatching season of 1914 an inexperienced man was in charge of the incubation and rearing. He had very poor success, and the Barred Plymouth Rock pullets available for the laying houses in the fall were relatively few in number, of a relatively late average date of hatching, and poorly grown. It is remarkable, not that the mean winter production was lower in 1914–15 than in 1913–14, but rather that it was so high as it was, taking all the circumstances into account. It appears that the system of breeding which made the selections on a progeny test basis was immediately and, to date, continuously effective.

4. That selection on a progeny test basis was effective is demonstrated not only by the general flock averages, but also by the fact that it was possible to propagate separately high and low producing strains. The high producing strains differed widely from the low producing in mean winter production. Taking the average for seven years in the case of the high, and four years in the case of the low, it appears that the mean winter production of the high producing strains was approximately two and a half times that of the low producing strains. At the end of the laying year 1911–12 the low producing lines were dropped. In the next year (breeding season of 1913) no birds were bred which were known to belong to segregating lines. Of course some were included which proved afterwards to have been segregating, but this fact could not, in any such case, have been told in advance from the records in hand. The propagation of low producing strains was attended with a great deal of practical difficulty with the environmental conditions under which one has to operate at this station. The growing season is short. In order to grow properly a pullet for laying purposes it is necessary that she be hatched after April 1 and before June 1 *at the latest,* and preferably before May 15. If, however, one selects birds which produce no eggs whatever before March 1, and use up some valuable time before they get well started in the spring cycle of laying it becomes perfectly clear that one is automatically prevented from getting any considerable number of early hatched chicks from such mothers. If late hatched chicks are used the results obtained as to winter production later will not be critical. These circumstances make the propagation of a low producing strain on a large scale really a difficult proposition. There is of course no difficulty in breeding birds which will not be winter layers. One only needs to hatch in June, July or August. But such birds will furnish no critical evidence regarding the inheritance of winter production.

5. The mean winter production for whole flocks over the entire period of the investigation is 35.05 eggs. In

the writer's opinion, based upon rather extended experience in the study of egg records, approximately this figure may be taken as representing the general average winter production of mixed flocks of Barred Plymouth Rocks (or of American birds generally with the probable exception of White Wyandottes), which have been hatched at the proper time and well reared. As evidence on this point the data presented in Table II have pertinent bearing. These data give the mean winter production of birds of the different American breeds obtained in the Fourth Philadelphia North American International Egg-Laying Competition, carried on at the Delaware Agricultural Experiment Station in 1914–15. The conditions under which these records are made are such as to safeguard their essential accuracy. The figures here given are the mean productions per bird up to the end of the eighteenth and seventeenth week of the laying after November 1, 1914. Owing to the fact that the original records as published are compiled by calendar weeks it is not possible to give the exact production from November 1 to March 1. Eighteen weeks gives 5 days laying over this period, and seventeen weeks gives 2 days under. Both sets of means have therefore been tabled. It should be said that the birds were kept in flocks of 5 birds each, thus tending to the most favorable condition for high individual records.

TABLE II

MEAN WINTER PRODUCTION OF FOWLS OF THE AMERICAN BREEDS, CALCULATED FROM RECORDS OF THE FOURTH INTERNATIONAL LAYING COMPETITION, 1914–15

Breed	No. of Birds	Mean from Nov. 1 to Mar. 5	Mean from Nov. 1 to Feb. 26
Barred Plymouth Rocks	45	29.20	25.47
All Plymouth Rocks	80	32.39	28.39
All Wyandottes	55	48.20	44.00
All Reds	75	38.37	34.42
All American breeds	210	38.67	34.63

From this table it is clear that the records presented in Table I average about the same as those of the 210 birds of the American breeds in the Delaware competition.

The Wyandottes alone give a distinctly higher mean, and this the writer has also found to be true of Irish egg records. The Barred Rocks in the competition this year give a somewhat abnormally low winter mean. Irish records[6] give a winter mean for 48 Barred Rocks, in flocks of 6 each, of 36.54 eggs per bird which is more nearly normal.

III

Let us now turn to the question of the interpretation of the data set forth in the preceding section. Broadly speaking what the facts gleaned from this seventeen-year experiment show is that mass selection for egg production was *not* effective, while selection which was based upon the performance of the progeny *was* extremely and quickly effective. What is the explanation of the difference? The facts are purely objective realities, about which dispute or question is idle. They are real and obvious matters. Regarding the interpretation of such facts as these there have been, and no doubt will continue to be for some time to come, differences of opinion amongst biologists. Under these circumstances, the writer will, then, with all respect and consideration for the differing opinions of others endeavor to make clear the view of the meaning of these results which he has come to hold.

In the first place we may definitely put aside any interpretation which bases its explanation of the results on environmental action. In an earlier paper the writer[7] has shown in detail the impossibility of this explanation for the results during the period of mass selection (the descending limb of the curve). The totality of the results here presented make it still more apparent that such an explanation can have no place here. We should have to suppose that the environmental influences were adverse throughout the period of mass selection, but suddenly became favorable when the method of breeding was changed, and have ameliorated at an ever increasing rate

[6] Murphy, L., *Jour. Dept. Agr. Ireland*, Vol. XIV, pp. 8–30, 1913.

[7] Pearl, R., Me. Agr. Expt. Stat. Ann. Rept., 1911, pp. 113–176.

as time has gone on. Nothing of the sort was, in fact, the case. The only explanation which can satisfy the case is one which is based upon or at least takes full account of the changing genetic constitution of the flock.

It appears to the writer that the essential key-note to the explanation of the results of this long experiment is found in the fact that *phænotypic variation of the character fecundity, in fowls, markedly transcends, in extent and degree, genotypic variation.* It is quite impossible in the great majority of cases to determine with precision what is a hen's genetic constitution with respect to fecundity from an examination of her egg record *alone.* In this case, as in so many others, but in an unusually pronounced degree, where the phænotypic distributions overlap, a sure diagnosis of genetic constitution can only be made by means of the progeny test. Lacking this the phænotypic performance becomes an always uncertain and at times very misleading guide.

It can be shown that if, during the period of mass selection, all the hens used as breeders had been, as they were supposed in the theory of the originators of that part of the experiment to be, either Type 1 or Type 2 females ($fL_1L_2 \cdot \widehat{Fl_2}l_1$ or $fL_1L_2 \cdot \widehat{Fl_2}L_1$) then the continued mass selection must have resulted in improvement. The *only* criterion of constitution which was used, however, was the bird's performance. But, taken by this criterion alone, there would be constantly chosen a proportion of birds whose genotype was for mediocre fecundity, but which made a performance record (phænotypic) sufficiently high to be selected. That this is what actually happened is evident from the curve (Fig. 1), but the fact was experimentally proved in 1909.[8]

Put in the fewest words, then, the reason why no effect was produced during the first ten years of selection and a marked effect was produced during the last seven, was, in the writer's opinion, because genotypically high producers were *uniformly* selected (in the high lines) during

[8] Pearl and Surface, Me. Agr. Expt. Stat. Ann. Rept., 1909, pp. 49–84.

the latter period, and were *not* uniformly selected in the former. By the introduction of the progeny test as an essential part of the selection the whole process of the creation of a highly fecund race of hens was transferred from the realm of blind chance to that of precise and definite control. And it becomes increasingly clear that mass selection, on the basis of performance (phænotypic appearance) alone, is in its essential nature a blind and haphazard process, no matter with what precision and stringency it is carried out, just so long as the correlation between the gametic and somatic conditions of the character selected is not perfect. And it is an outstanding result of the Mendelian investigations of the last 15 years that the gamete-soma correlation is very rarely, if ever, perfect.[9]

It appears on this view that selection for high egg production in the fowl is effective when it is real. That is, if one selects *genetically* high producers by means of the trap-nest *plus* the progeny test, he succeeds very rapidly in fixing a high producing strain. If on the other hand he merely selects high layers by the trap-nest record alone, he is not really selecting genetically high producers except in a portion of the cases. Under these circumstances he makes no progress in building up a highly fecund strain. To be effective in changing the average productiveness of a flock of poultry selection must pick out those birds as breeders which carry the factors for high fecundity genetically, *i. e.*, as an integral part of their hereditary make-up, *and not any other birds.*

With the above interpretation of the results of seventeen years' continuous selection of the character fecundity, all the facts known to the writer are in complete accord. No other interpretation of the results of this experiment has yet been suggested which will meet all the facts.

[9] Complete citations on this point would make a tolerably full bibliography of Mendelism. The methodological or the strictly quantitative aspects of the problem have been but little dealt with. In this connection cf. W. F. R. Weldon, *Biometrika*, Vol. I, pp. 228–254, 1902, and R. Pearl, *Biol. Bulletin*, Vol. XXI, pp. 339–366, 1911.

IV

What bearing have these results upon the general problem of the effectiveness of selection in modifying germinal determiners? Let us at the outstart endeavor to be quite clear as to the problem. It has been shown in what has preceded that in the long experiment a change in the average condition of the population has occurred, and has been coincident with selection. The writer has no hesitation in saying that the increase in average productiveness since 1908 has been *caused* by the particular kind of selective breeding practised. Furthermore the average productiveness at the present time transcends any average known in the previous history of the stock.

Granting all this, however, as plain matter of fact, it does not, in the writer's opinion, afford one iota of evidence that through the process of selection the hereditary determiners of fecundity either have been or can be changed. All that the selection has done, so far as we have any evidence, is to change the constitution of the *population* in respect of fecundity genotypes. There is no evidence that the genotypes themselves have been changed. On the contrary, everything indicates that they have not been changed. During the last seventeen years we have merely sorted out from a mixed population, by a systematic method of breeding, those individuals which were alike in one respect, and have sold all the rest to the butcher. That one respect was that each individual bore progeny which were high producers.[10] Those individuals chosen as breeders in 1908 and 1914 were precisely alike in this particular There were more of them available in proportion to the whole flock in 1914 than in 1908, but, as individuals, I am unable to discern any particular in which they were different in 1914 from what they were in 1908.

The general point here involved is essentially the same as one with which we are more familiar in demographic

[10] I am, of course, referring to the high line selections only, merely for the sake of verbal economy. The same reasoning *mutatis mutandis* applies to the low lines.

statistics. The constitution of a population does not directly affect the individual. My expectation of life will not be materially increased if I chance to move into a community in which all the other inhabitants are of advanced age. To this everyone will agree. But the extreme selectionist appears to believe that in some mysterious way the act of continued selection, which means concretely only the transference of each selected individual from one cage or pen to another to breed, will in and of itself change the germ-plasm, so that after the act it is different from what it was before! It does not seem that the evidence that such is in fact the case is critically valid. A careful study of the very interesting and valuable work of Castle and Phillips[11] with rats leaves the writer with the feeling that those experiments prove no more than do the experiments here reported: namely that the composition of a population may be altered by selection. It does not appear to be proven that selection has essentially altered the constitution of the germ-plasm of any particular individual as compared with the germ-plasm of that individual's ancestors, making due allowance of course for the phenomenon of segregation. That selection can alter the composition of a population with respect to genetic determiners, by a process of sorting over what is already there and rejecting some portion, no one can doubt. But it still appears to the writer to be true that: "It has never yet been demonstrated, so far as I know, that the absolute somatic value of a particular hereditary factor or determinant (*i. e.*, its power to cause a quantitatively definite degree of somatic development of a character) can be changed by selection on a somatic basis, however long continued."[12]

[11] Castle, W. E., and Phillips, J. C., "Piebald Rats and Selection," Carnegie Institution of Washington Publication No. 195, 1914.

[12] R. Pearl, *Jour. Exp. Zool.*, Vol. 13, p. 264, 1912.

SPECIFIC AND VARIETAL CHARACTERS IN ANNUAL SUNFLOWERS

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THE group of *Helianthus annuus,* the typical, annual sunflowers of North America, is not a large one. The annual habit seems to have been acquired independently by several different Helianthine stocks, so that *H. bolanderi* Gray, *H. exilis* Gray, *H. floridanus* Gray and *H. tephrodes* Gray are to be excluded from the *H. annuus* group. The subgenus *Helianthus* s. str., or *Euhelianthus,* contains the following:

1. *H. annuus* Linn. Based on the large cultivated form (*H. macrocarpus* D. C.), Dr. A. H. Church of Oxford has investigated the history of this plant, and I take the liberty of quoting from a letter he wrote on March 4, 1915:

> The published accounts of the giant sunflower in Europe in the sixteenth century are so precise that it is interesting to remark that this is in fact the *oldest* mutation known, which is *still* with us, quite unaffected, though still never quite a pure strain, owing to insect pollination. The facts are quite simple. The first description of the plant, by Dodonæus (1567), tells us it grew in the Botanic Garden at Madrid, *24 feet.* At the Padua Garden, *indoors,* in a viridarium or orangery, *40 feet!* The usual height was 20 ft. The first English specimens, grown in London by Gerard, were 14 ft.; and 15 ft. is the local record here. The giant form is known by carrying one *head,* and having no trace of axillary buds, =*Monocephalic* strain, as opposed to reverting branching individuals. . . . The next point is, where did it come from? *From Peru,* say the Herbals, but all Spanish things from America came *via* Peru, because this was the last port of call. Hence Mexico is regarded as the home. On the other hand *Ximenes,* who lived in Mexico several years, and *Hernandez* after him, call it the *Chimalacak del Peru;* "acak" I find means a *reed,* and thus refers to the long tall single stem of the cultivated crop. The inference is that the plant as we know it was evolved by ages of selection *in Peru,* by guano fed cultivation, possibly long before Inca rule, the plant having been taken by all migrating tribes from the Mexican district. . . . Regarded as a product of Peruvian agriculture the sunflower is curiously parallel with the *maize.* . . . It was the oil crop of ancient America.

The true *H. annuus* appears to be quite unknown in the wild state, but nevertheless the monocephalic character may have arisen among wild plants. Dr. Church makes the following suggestion:

If the monocephalic form is the giant of cultivation derived from the Prairie form, it should be possible *to repeat* the history, by growing Prairie forms in quantity, and selecting the suitable mutations when they appear under the stimulus of excess manure (guano for choice). My idea has been that, knowing what to look for, it might be possible to get somewhere near it in say 10 years; though the Indians possibly took 2,000. General structural evidence alone suffices to show that the monocephalic strain is the response to selection for close cultivation (about two plants per square yard). The solitary heads are required for simultaneous harvesting.

2. *H. lenticularis* Douglas. The prairie sunflower, much branched, and normally with dark disc. It has been regarded as the wild type of *H. annuus,* but Rydberg treats it as a distinct species. In crosses with typical *annuus,* the F_1 is intermediate, often with a tendency to fasciation. If *annuus* and *lenticularis* are considered specifically distinct, we have to face the difficulty that the former is known only in cultivation, and its one "specific" character, the monocephalous habit, is not constant.[1] The color of the disc is not a reliable distinction, since yellow discs occur in wild plants. Possibly the variation shown by *H. annuus* may be explained by contamination with *lenticularis,* since some strains, at least, are constant in their characters. At present, however, it seems probable that no wild *species* ever existed with typical *H. annuus* characters; the actual facts would probably be best represented by considering *lenticularis* the species, and *annuus* a cultivated variety derived therefrom. Since, however, the latter was first named, the species-aggregate will have to be called *H. annuus,* and the nomenclatural outcome will be as follows:

[1] Shull, *Botanical Gazette,* **45**, 105, figures a much branched form which is not the wild *lenticularis.*

Helianthus annuus L.
(*a*) *lenticularis* (Dougl.)
(*b*) *macrocarpus* (D.C.) = *annuus* L., s. str.

At the same time, for ordinary purposes, it may be permissible to simply write *H. lenticularis* when referring to the wild plant.

3. *H. aridus* Rydberg. Like *H. lenticularis,* but leaves lanceolate or narrowly deltoid, minutely toothed or entire. Montana to New Mexico. Nelson calls this a synonym of *H. petiolaris,* which it certainly is not. It must be called *H. lenticularis aridus* (Rydb.) or *H. annuus lenticularis* var. *aridus,* since it is a variable form of *lenticularis,* which may possibly be due to crossing with *H. petiolaris,* the hybrid having crossed back with *lenticularis.* From the mode of its occurrence it is nearly certain that it is not a simple *lenticularis* × *petiolaris* hybrid, *petiolaris* being often absent from the immediate vicinity.

To give an idea of the actual condition of affairs where *H. aridus* occurs in Colorado, I present a synopsis of the forms found at Longmont, August 30, 1914:

(*A*) *H. aridus* type; smaller and more slender, with cuneate bases to leaves.
  (*a*) Variety with yellow disc; two plants found.
    Disc 19 mm. diameter, light yellow (corollas entirely dull light yellow); rays ordinary; foliage unusually pale; base of leaves rather broad-cuneate, marginal teeth feeble.
  (*b*) Disc dark (corolla lobes dark reddish). Leaves with cuneate base and subentire margins; typical *aridus.* Involucral bracts *very* broad and bristly. These rather slender plants have small discs (17–21 mm. diam.) and very ample rays, which are not very numerous (10–13); color of rays rich orange yellow; stems lightly speckled with purplish.
    (i) Rays longer, about 38 mm. long and 15 broad.
    (ii) Rays shorter, about 28 mm. long and 14 broad.
    (This difference in size of rays is probably environmental.)

(*B*) *H. lenticularis* type; bases of leaves truncate or cordate; plants usually more robust; disc dark.
  (*a*) Aspect of *aridus,* being rather slender, with small (diam. 23.5 mm.) disc and long rays; but leaves broadly truncate at base and rather strongly toothed, *quite lenticularis* style. This is a very pretty form, with long rays (about 40 mm. long and 11 broad), more or less twisted at end, and rather narrow. The rays number about 15.

(b) Aspect of *lenticularis;* more or less robust, rays rather short and numerous.

(i) Rays comparatively short and broad (about 22 mm. long and 10 broad on a small head), the middle third beneath with its apical half variably light brownish-red. Leaves thick, with broad petioles.

(ii) Rays normal.

(α) Upper leaves ovate, scarcely at all dentate, inequilateral. Disc small (22 mm. diam.), rather paler than usual, the corolla lobes showing less red. Rays 14, about 31 mm. long and 11.5 broad.

(β) Upper leaves broad at base, but somewhat cuneate, rather feebly dentate. Disc 20–23 mm. diameter. A small head (disc 20 mm.) with many (21) quite *short* rays, about 17 mm. long and 7 broad.

(γ) Typical *lenticularis,* with broad-based strongly dentate leaves. Disc 37 mm. diameter; rays 37 mm. long, numerous (about 33).

It would of course be possible to maintain that *H. aridus* was originally a distinct or isolated species, which has now lost its purity by crossing with *lenticularis.* We can at least say this, that if *annuus, lenticularis* and *aridus,* in their pure forms, inhabited three different islands, few would hesitate to regard them as perfectly "good species." Also, if they grow mixed for any length of time, they are sure to suffer from "vicinism" to such an extent as to lose their supposed original distinctness. At present, however, we have no assurance that *H. aridus* has ever constituted a distinct species, in the sense of occupying any considerable area in its pure form. On the other hand, it is manifestly not a "fluctuating variation," due to mere environmental conditions.

4. *H. petiolaris* Nuttall. Described by Nuttall in 1821, from "the sandy shores of the Arkansa," and recommended as "an ornamental annual of easy culture." It extends from British America to the State of Chihuahua. It differs from *H. lenticularis* by (*a*) smaller stature, (*b*) leaves differently shaped, lanceolate or broad-lanceolate, not dentate, more or less shiny above, those of *lenticularis* being quite dull, (*c*) bracts of involucre lanceolate, with margin very short-ciliate. Stem rough, with a little purplish color; basal third of rays deeper orange than the rest.

This is a good species in the ordinary sense; in Colorado it is often found abundantly in the cañons of the foothills, growing without admixture of other species. Lower down, it frequently occurs with *lenticularis*.

The variety *patens* (Lehm.) Rydb. is said to differ by having the heads larger, long-peduncled, the peduncles fleshy toward the top; leaves large, long-petioled. Nuttall described his original *petiolaris* as having the peduncles "of great length," and the petioles "of an extraordinary length," though the leaves were "rather small." Probably *patens* is not far from the original *petiolaris*. Gray considered *patens* a synonym. According to Rydberg, the leaves of *patens* are broadly ovate or subcordate, much in the style of *lenticularis*, while the bracts are those of *petiolaris*, thus reversing the condition of *aridus*. It is possible that *aridus* and *patens* are both remote results of the *lenticularis* × *petiolaris* cross, but in the vicinity of Boulder, when *aridus* is common, I have not found *patens*.

5. *H. canus* (Britton) Wooton and Standley. A species of New Mexico, Chihuahua, and adjacent regions, close to *petiolaris*, but with abundant white pubescence on leaves and stems. The involucral bracts are of the *petiolaris* type. This is to *petiolaris* much as *H. argophyllus* is to *lenticularis*, but the pubescence is long and spreading, not subappressed and silky.

6. *H. argophyllus* Torrey and Gray. Discovered by Drummond in dry soil in Texas. This has the form and leaves of *lenticularis*, but is very remarkable for the long subappressed silky white hairs, totally different from those of any other *Helianthus* known to me. Gray remarks that it " degenerates in cultivation apparently into *H. annuus*," which merely means that it suffers from vicinism. Old cultivated stocks, kept pure, are quite constant. A remarkable feature of *H. argophyllus* is the extremely slow growth, at least until near flowering time. This peculiarity is dominant in a cross with *H. annuus* × *lenticularis*.

7. *H. debilis* Nuttall. Florida to Texas.

8. *H. praecox* Engelm. and Gray. Florida to Texas, near coast. Differs from *debilis* by being strongly hirsute.

9. *H. cucumerifolius* Torrey and Gray. Texas. Differs from *debilis* and *præcox* by having the branches mottled with purple.

The last three were eventually reduced by Gray to a single species, but Small keeps them separate. My wife and I have grown *H. cucumerifolius* for several years, and have crossed it with *annuus* × *lenticularis*. The first cross is quite fertile, but it is impossible to get any quantity of F_2 seed. Mr. Leonard Sutton in England has had the same experience; he writes (April 3, 1915)

> We are arranging for a large breadth of the *cucumerifolius* crosses this season, but we have found as you mention that very little seed is produced, and we are hoping that the plant will improve in this respect if grown for a few years, and the best seeding plants are selected for stock.

These hybrids are of considerable horticultural value, especially those derived from crosses with the red sunflower, so it is desirable to secure fertile strains if possible. Something may be attained by crossing back with the parent species.

The *H. cucumerifolius* type is dwarf, freely branching, with broad bright green leaves, shiny on both sides. The involucral bracts are long and narrow. The bulb or swelling of the disc corollas is minutely puberulent, whereas that of the *lenticularis* forms is long hairy. In the F_1 hybrid the bulb is long-hairy as in *lenticularis*, the character being dominant. Although *H. cucumerifolius* is very unlike the other species (except *debilis* and *præcox*) in apperance, its constant structural differences are very few. The base of the leaves, as in the *annuus* forms, may be auriculate or truncate. The disc bracts may be long-ciliate, or with the margins merely appearing scurfy. It is proper to state that my material belonged to cultivated strains; possibly the wild plant is less variable.

Thus we have at the most nine species, which can probably be reduced to five. They belong to the region which used to be marked in old geography books as the "Great American Desert," though members of the *debilis* group extend along the Gulf States to Florida. The dominant, widely distributed form is *lenticularis*, a plant of sandy river-bottoms and similar places, which has spread as a weed in cultivated areas. Prior to the era of cultivation it is probable that *H. petiolaris* occupied a greater area, at least in acreage. At the present time *H. lenticularis* is common in California, but I suspect that it has been introduced into the Pacific coast region by man.

In order to give an idea of the cultivated forms of our group, I have made a table from Sutton's Catalogue for 1915.

	Silver-leaved (*argophyllus*-type); rays yellow; disc black; 5 ft.	*Silver-leaved.*
	Not silver-leaved	1.
1.	*Cucumerifolius*-type, none over 4 ft. high	2.
	Annuus-type, mostly tall	6.
2.	Only 12 inches high, compact	*Dwarf Miniature.*
	Four feet high	3.
3.	Rays rolled, like the cactus dahlia	*Orion.*
	Rays not rolled	4.
4.	Rays pale primrose, disc dark	*Primrose Stella.*
	Rays bright yellow	5.
5.	Heads small, with dark disc	*Miniature.*
	Heads larger, rays long	*Stella.*
6.	Rays wholly or partly chestnut red	*Red.*
	Rays wholly or partly vinous	*Langley Gem*
	Rays primrose	7.
	Rays bright yellow or orange	9.
7.	Double (i. e., disc florets ligulate)	*Double Primrose.*
	Single; disc black	8.
8.	Tall, 6 ft. high	*Primrose Perfection.*
	Dwarf, 2.5–3 ft.	*Single Dwarf Primrose.*
9.	Double (*i. e.*, disc florets ligulate)	10.
	Single (*i. e.*, normal heads)	11.
10.	Flowers orange; 6 ft.	*Double* (also a double 5 ft. high).
	Compact habit; 3 ft.	*Dwarf Double.*
11.	Heads extremely large; height 8–10 ft.	*Giant.*
	Heads ordinary or smallish; disc dark; height 6 ft.	12.
12.	Heads medium size	*Æsthetic Gem* (J. Veitch & Sons).
	Early flowering	*Earliest of All.*

Of the above *Red* is *coronatus,* and *Langley Gem* is *vinosus,* both derived from our Boulder cultures. The seed offered as Langley Gem was grown in Boulder. The *Primrose* variety I have called *primulinus.*

An old cultivated variety is *H. annuus* var. *indicus* (*Helianthus indicus* Linn., Mant. I, 117), peculiar for the foliar expansion of the involucral bracts. It did not come from India, but from Egypt. *Tithonia speciosa,* once regarded by Hooker as a *Helianthus,* has the bracts normally foliaceous. In 1913 I witnessed the appearance of foliaceous bracts in the F_2 generation from *primulinus* × *coronatus.* The plant in question was a very abnormal dwarf, wholly unlike the rest of its generation, or any known parents. It was described as follows:

Dwarf, about 28 mm. high; slender, fasciated at top of stem; rays vinous, but on nearly all the heads a very dilute and dingy color; disc dark, stigmatic branches dark red; apical part of disc corollas dark greenish, tipped with red, and very hairy; anthers not projecting, but not shrivelled, almost wholly without pollen, and what there is probably no good; achenes hairy, usually with supernumerary pappus scales; pappus scales stained with pink; involucral bracts long and tapering, strongly hirsute, curled over, one or two outer ones long and foliaceous; stem hirsute; leaves long and narrow, narrowly cuneate at base; margins irregularly, sharply dentate, entire on small very narrow leaves; sometimes one of the large lateral veins of the leaves, and its supporting tissue, absent.

Such a plant may result from some unwonted combination of genes, whereby the normal constitution is broken down and in the resulting disruption characters usually suppressed appear. Such monstrosities quickly perish, but during their transient existence may reveal, like a drunken man, matters which in the well behaved would never reach the surface.

One of the most remarkable of cultivated varieties is the *Chrysanthemum-flowered,* of which we obtained a

perfectly constant and uniform strain from Dreer. It may be named var. *chrysanthemoides;* plants of the same general type have passed in horticulture as var. *californicus* (not *H. californicus* D.C.)

Helianthus annuus var. *chrysanthemoides*

Manner of growth.—(Tested in two seasons). Grows much more slowly than the other forms (except *argophyllus),* but is very robust. Nine plants studied were 15–17 inches high July 14, about 36 inches July 30, and coming into flower at about 5 ft., 6 inches, August 15.

Foliage.—At first (June 8) leaves are narrow and long; very uniform. Later, the upper (small) leaves are conspicuously pallid. At time of flowering the leaves are broad, cordate, with auriculate base; surface very strongly crinkled; margin moderately dentate.

Pubescence.—Leaves soft with very scanty pubescence; petioles somewhat scabrous; stems, especially toward the top and under the heads, with abundant and conspicuous soft white pubescence.

Heads.—Stalks greatly broadened under heads, diameter about 27 mm. just under bracts; involucral bracts hairy, the marginal hairs not longer than those covering the backs of the bracts, five strong veins, and others weak; basal half of bracts about 15 mm. broad, gradually, not abruptly, tapering to acuminate ends; bracts extending about 18 mm. beyond outer florets, which are like the inner ones; heads entirely double (*i. e.,* corollas ligulate), rays very bright orange or saffron, discs light green before they come into flower; immature achenes with much silvery hair.

This plant is so distinct, structurally and physiologically, that if it were not known to have originated in cultivation, it might well pass as a distinct species. Although I have no information concerning its history, I can only suppose that it is part *argophyllus.* Mr. Leonard Sutton writes me that the similar *Double* catalogued by him, which is of continental origin, does not grow more slowly

Fig. 1, above, Double Red Sunflower. Fig. 2, below, Helianthus von tortuosus.

than other sunflowers in its early stages. The var. *chrysanthemoides* was found by us to cross freely with the *annuus* × *lenticularis* varieties, producing a series of semi-doubles. The double and semi-double forms extracted from this cross and from crosses with Sutton's various double forms need not be described here, but in order to illustrate the double type, I give a figure of a full double with chestnut color, the red being derived from the variety *coronatus* (Fig. 1).

Modifications of the Rays

Number.—Halsted (Rept. Bot. Dept. N. J. Agric. Exp. Sta. for 1911, pp. 335–337) has given elaborate data from the branched form of the cultivated *H. annuus*, showing that the terminal heads have most rays, and when there are many lateral branches, the rays on these are comparatively few. Nevertheless, there are inherited differences in the number of rays, not depending on conditions of nutrition. I observed a striking case by the roadside in Boulder, where three wild *lenticularis* plants, growing close together, differed thus:

(*a*) Rays normal, at right angles to axis; number of rays in well-formed heads, 21, 21, 21, 21, 20, 21, 21.

(*b*) Rays normal, elevated, their plane oblique in relation to the axis of head; rays in well-formed heads, 14, 13, 14, 14, 14, 11, 15.

(*c*) Rays set obliquely, but less so than in *b*; rays in well-formed heads, 18, 18, 18, 19. In this plant many of the rays were modified by quilling and splitting, some being completely quilled, *i. e.,* hollow and tubular. The normal rays were very obtuse, and distinctly emarginate at end. Some showed a little red color on apical part of middle third beneath.

Length.—The length also differs, the differences due sometimes to race, sometimes to illumination or nutrition. In our cultures mutational forms have arisen with unusually short rays, thus:

(*a*) Var. *vinosus* with disc 55 mm. broad, rays only 35 mm. long; disc unusually convex.

(*b*) Var. *bicolor* with disc 64 mm. diameter, rays only 29 mm. (Next to it, in the same lot, grew a plant with disc diameter 38, rays 47 mm.)

These measurements represent average heads from the respective plants. A quite analogous variation was seen in two plants of *Ratibida columnifera,* growing at Boulder along with the typical form (var. nov. *breviradiata,* rays yellow, only about 10 mm. long, about half the normal size).

Torsion.—A peculiar form which appeared in our cultures is the variety *tortuosus,* in which the ends of the rays are twisted, as though in curl papers. This is wholly unattractive, but other variations have the long rays moderately curled or twisted, promising the development of a series of forms analogous to the cactus dahlias. As with the cactus dahlias, the rays may be rolled instead of twisted; a wild form of this type may be described thus:

Helianthus lenticularis var. n. *angustus.* Rays about 20, narrow, rolled, so that they are separated by wide intervals. The rays were 36 mm. long and 5 wide (a normal *lenticularis* ray 30 mm. long is 9 wide). Disc 26 mm. diameter. Goodview, Colorado, July 28, 1913.

Tubular Rays.—Under the heading "Number" above, a case of completely quilled rays in a wild sunflower is recorded. This peculiar modification indicates some deep-seated tendency in the Compositæ, since it appears in several genera, *e. g.:*

(*a*) *Ratibida columnifera* var. n. *tubularis.* Rays of the usual orange color, about 25 mm. long and 3.5 broad, completely quilled, being hollow cylinders. Flagstaff Hill, Boulder, Colorado, July 19, 1914.

(*b*) *Rudbeckia hirta* var. *tubuliforme* S. H. Burnham Amer. Botanist, Feb., 1914.

(*c*) *Gaillardia pulchella* var. *fistulosa* (*G. fistulosa* Hort.).

Emarginate and Cleft Rays.—This is another common modification, also observed in other genera, as *Ratibida* (*R. columnifera* var. n. *incisa;* rays with one or two deep

incisions, and also some narrow supplementary rays; Boulder, Colorado, August 8, W. P. Cockerell).

Double Rows.—The disc remaining normal, the rays may be in two rows, indicating an approach to a type resembling the star dahlias.

Color.—The yellow may be of various shades from deep orange to very pale, approaching white. This has already been discussed in *Science,* August 21, 1914, pp. 283–285. It may be possible eventually to get a pure white. Dr. Church (in litt.) refers to a white form as having been mentioned long ago by Hernandez. There is also the development of the soluble (anthocyanin) red pigment, giving us the chestnut red and wine red varieties.

Conclusions

It is impossible at the present time to give all the evidence on which opinions have been formed, but such facts as are reported above, and others, seem to suggest the following generalizations:

1. The number of genes or determiners in *Helianthus* is not infinitely great; it is probably very much less than exists in most animals, and the study of the processes of heredity is relatively simple.

2. In the history of the sunflowers of the *H. annuus* group, there have been few really new developments. Species which seem very distinct prove on examination to have few special characters of their own.

3. It is quite common for variations to arise, in wild and cultivated plants, which appear to break the type, and initiate something altogether new. When, however, we begin to gather data on the variation of the Compositæ, we find that practically all these "new" variations repeat themseles in various species, and at various times, indicating that they represent deep-seated common tendencies. Their occurrence among wild plants shows that they are not necessarily connected in any way with cultivation, and it is equally evident that they need not indi-

cate any sort of hybridization. For example, *Ratibida columnifera* presents many variations parallel with those of *Helianthus,* in localities where it is the only species of its genus.

4. We are led, then, to think of the annual sunflowers as plants representing a certain complex of potentialities or genes (of which we may hope at length to make a reasonably complete catalogue), offering these in different combinations at different times, usually failing to register any permanent advance, but once in a long while reaching a new position of stability, suited to a particular environment. These positions of stability represent what we call the species. As with the dahlia, the horticulturist may expect to be able to produce many interesting varieties by selecting and saving the various possible combinations, but analysis shows that the genes going into these are the old ones, the effects of which may be seen from time to time even in wild plants.

The perennial sunflowers appear to offer a more complex problem. Mr. S. Alexander has found hundreds of what are considered "elementary species" in Michigan. He has been good enough to send me a large number of these, and I can testify that they are appreciably different; yet they seem to represent recombinations of old characters, already known to exist in the species of the manuals. Some would dismiss them, along with the multitudes of *Cratægus,* as hybrids; but it does not seem justifiable to assume hybridization without better evidence. We have sufficient proof, I think, that all sorts of new combinations of characters may arise within a type, without hybridization.

Undoubtedly new determiners are formed (how, we need not here speculate) from time to time, but the occurrence must be so rare and so difficult to demonstrate that we can hardly hope to obtain satisfactory evidence concerning it.

THE INHERITANCE OF DOUBLENESS IN MATTHIOLA AND PETUNIA. I. THE HYPOTHESES*

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The peculiar inheritance of "doubleness" in stocks (*Matthiola*) has long been a matter of special interest. Some races produce only single-flowering plants. A pure double-flowering race, on the other hand, is an impossibility; the doubles are absolutely sterile, stamens and pistils

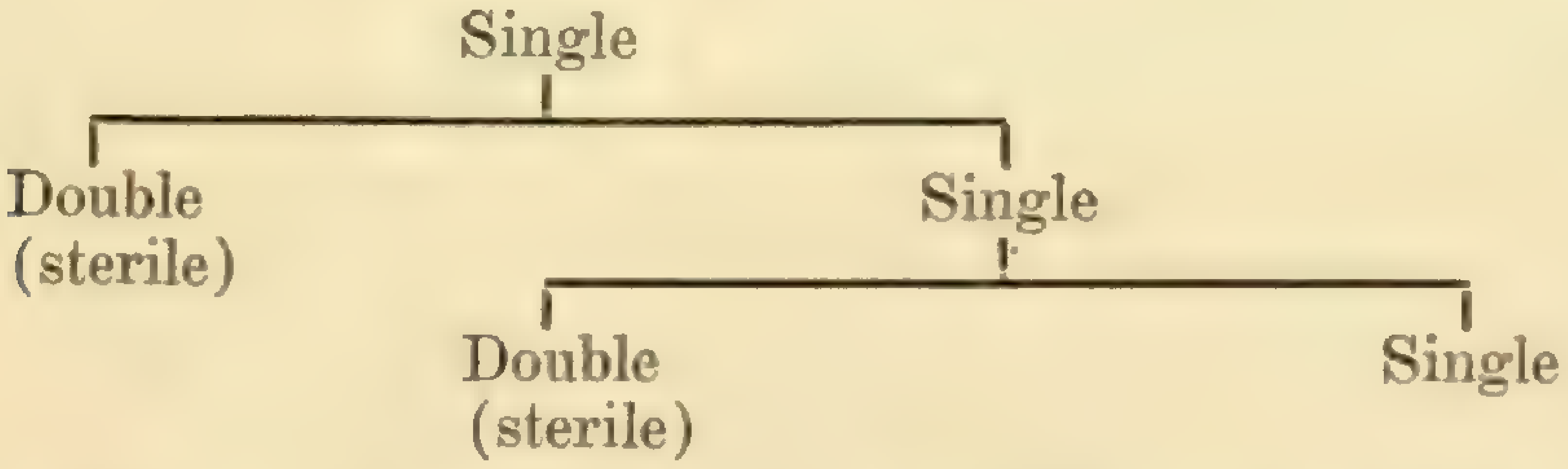
Fig. 1. *Matthiola* plants, unselected progeny of two parents, showing the mixture of singles and doubles invariably given by this variety ("Snowflake"). Note that the singles, in these cool-greenhouse cultures, differ little from the doubles in earliness of flowering. (The singles and doubles have been separated for photographing. The singles are plants 1, 2, 3, and 11 (misplaced), from the left side, in the upper row, and plants 1 to 5 in the lower row.)

being entirely absent. Certain races, however, consist of both singles and doubles, in nearly equal numbers,[1] each generation being descended from the singles of the pre-

* Paper No. 17, Citrus Experiment Station, College of Agriculture, University of California, Riverside, California.

[1] The usual proportion of doubles in large cultures seems to be near 53 per cent., or perhaps slightly higher in some cases.

ceding generation (see Fig. 1); the following diagram shows the mode of inheritance in such races:



Miss Saunders (1911; 1913; Bateson, 1909, pp. 201–204) has done a great amount of work on heredity in *Matthiola*, and has developed an ingenious hypothesis to explain the peculiar behavior of doubleness. Goldschmidt (1913) has given another explanation, which has been vigorously criticized by Miss Saunders. Several years ago (perhaps in 1909), largely on the basis of Miss Saunders's evidence, I formulated a hypothesis somewhat simpler than either of those just mentioned.

In view of the special interest of the case at present, and the fact that one or both of the essential points of my explanation have been suggested incidentally by another writer (Belling, 1915, 1915a), it seems desirable to give a general review of the hypotheses at this time.[2]

As Miss Saunders's (1911; Bateson, 1909, p. 201–204) crosses have shown, the "double-throwing" singles are heterozygous, the approximately 1:1 ratio being due to the fact that the functional pollen is all double-carrying. This is shown by Miss Saunders's crosses between double-throwers and pure singles. When the double-thrower is the seed-parent, about half the F_1 progeny are heterozygous, the rest being pure singles; about half the double-thrower eggs, then, are "double-carrying." On the other

[2] My own data bearing on the problem have largely been published (Frost, 1911) or will be published in two papers (Frost, unpublished) soon to appear; some further evidence, relating to the proportions secured with some 8,000 plants of one variety, together with a summary of my other data, is to be presented in a paper to follow the present one. Aside, however, from one important general feature of these results, to be briefly stated below, the view given here is dependent on Miss Saunders's evidence and that cited by her.

hand, when the double-thrower is the pollen-parent, *all* the F_1 progeny are heterozygous; hence all the double-thrower-pollen is double-carrying. These facts are illustrated by the two following diagrams (adapted from Goldschmidt):

P_1 Double-thrower ♀ — Pure single ♂
F_1 Single (½) | Single (½)
F_2 Single | Single (¼) | Single (½) | Double (¼)
F_3 Single (pure) | Single (pure) | Single (¼) (pure) | Single (½) (heterozygous) | Double (¼) (sterile)

P_1 Pure single ♀ — Double-thrower ♂
F_1 Single
F_2 Single (¼) | Single (½) | Double (¼)
F_3 Single (pure) | Single (¼) (pure) | Single (½) (heterozygous) | Double (¼) (sterile)

In these two crosses, *where the "singleness" in the F_1 (or later) heterozygotes comes entirely from the pure single parent,* we get what seems to be an ordinary Mendelian[3] result in F_2; the pollen of these heterozygotes must carry both "singleness" and "doubleness." The absence of singleness from the double-thrower pollen is taken by Bateson (1914, p. 292, foot-note) as almost conclusive evidence of somatic segregation of factors, occurring in such a way that the pollen-mother-cells receive only doubleness. Neither he nor Miss Saunders, however, gives any reason why singleness, rather than doubleness, should be thus eliminated. Goldschmidt (1913) and Belling (1915, p. 126) have stated that selective sterility of pollen will also explain the case, and definite evidence for this view is presented below.

[3] In some crosses the proportion of doubles is smaller, possibly 1/16 instead of 1/4.

To explain the slight but constant excess of doubles over singles, Miss Saunders assumes that two complementary linked factors, X and Y, are essential to singleness, and that these factors cannot be carried by the male gametes of the double-throwers, which are all xy. X and Y are supposed to be so linked in the ovules that the four kinds of eggs are produced, not in equal numbers, but in the ratio 7XY : 1Xy : 1xY : 7xy—or else in the ratio 15 : 1 : 1 : 15. Fertilization by xy pollen will give, in the former case, $7XY \cdot xy + 1Xy \cdot xy + 1xY \cdot xy + 7xy \cdot xy$; if only zygotes having both X and Y are single-flowering, only the first class will consist of singles, and the doubles will constitute 9/16, or 56¼ per cent., of the total. Linkage on the 15 : 1 plan would give 17/32, or 53⅛ per cent., of doubles.[4]

For certain cases where crosses with pure singles have given much less than 25 per cent. of doubles in F_2, Miss Saunders assumes the presence of a second set of two linked factors, X′ and Y′; then any zygote receiving X or X′ together with Y or Y′ is a single, and the proportion of doubles is correspondingly reduced.

Von Tschermak (1912) favors Miss Saunders's hypothesis; he suggests the possibility of selective elimination (in a dihybrid scheme), but does not consider this explanation probable. It would seem, however, in view of considerations stated below, that any dihybrid scheme to explain the usual slight deviation of the double-throwing races from a 1 : 1 ratio is unnecessarily complex.

Goldschmidt's (1913) hypothesis assumes selective degeneration or sterility of pollen in the double-throwers, and considers the case to be one of sex-linkage, classing the slightly aberrant ratio with the known cases of slight deviation in the sex-ratio in animals. He supposes that this "hermaphroditic" plant is homozygous for a distinct factor for femaleness (F), producing eggs all of which carry this factor. He assumes that singleness is

[4] Miss Saunders (1911) rather favors the latter gametic ratio, which also corresponds closely to my own data.

determined by one dominant factor, S; the eggs of the double-thrower, then, are SF ("single") and sF ("double"). He assumes, also, that half the pollen-grains or microspores, in all races of *Matthiola,* lack F, probably because of elimination of part of an X-chromosome, and *that these pollen-grains degenerate* or at least are non-functional, so that no staminate plants are produced. It is necessary to assume, then, that in the double-throwing races S (or s) and F are carried by the same chromosome, and that the S-carrying chromosome is always the one to eliminate F. The S-carrying chromosomes will then be the ones destined to degenerate. The pollen resulting is of two kinds, Sf ("single," non-functional), and sF ("double," functional).

The double-throwing plant, then, is SFsF; its eggs are SF and sF, while its pollen-grains are Sf (non-functional) and sF. Self-pollination gives, then, SFsF (heterozygous or double-throwing singles) and sFsF (homozygous sterile doubles).

The factor S, however, can not in itself, in general, insure pollen-degeneration, since homozygous singles (SS) produce fertile pollen. Nor can the case be one of degeneration of all pollen-grains receiving *a maternal X-chromosome,* as is proved by the results of crossing SS and Ss races. Heterozygous singles (Ss) which get the S factor from a pure single (SS) parent, either through egg or through sperm, produce good S pollen, as is shown by the ordinary Mendelian ratio among their progeny (1 homozygous single (SS) : 2 heterozygous singles (Ss) : 1 homozygous double (ss). Goldschmidt is driven to assume, therefore, that the singleness factor (S_1) in the double-throwers differs from that in the pure singles (S)—or else to suppose that another factor interferes in the former type.

It will be seen that Goldschmidt gives, at most, only an indefinite implied explanation of the deviation of the double-single ratio from equality in the double-throwing races. And it is hard to see what advantage is secured

by introducing sex-factors into the discussion at all, when all actual individuals have both stamens and pistils, or else neither. When we assume—as Goldschmidt does—that the factor S is so modified in the double-throwing races as to insure the sterility of pollen-grains receiving it, the known facts must follow; it seems wholly superfluous to refer the sterility to linkage of S_1 with a sex-factor. The hypothesis seems quite unnecessarily complex; there is no real evidence here for the existence of a distinctly heritable femaleness factor, or for any elimination of sex-factors in pollen-formation, or for the occurrence of non-functional pollen in ordinary pure single (SS) races.

In a reply to Goldschmidt, Miss Saunders (1913) gives a very clear presentation of both her formulation and that of Professor Goldschmidt, urging most of the objections to the latter scheme which are stated above, but especially emphasizing its failure to explain the excess of doubles over 50 per cent. She also objects to the assumption of the existence of non-functional pollen, but I can not agree with her on this point.

I have sectioned anthers prepared for cytological study, and have frequently observed stages subsequent to the reduction divisions. The spore-tetrads appear normal, and there seems to be no early and conspicuous evidence of later degeneration. The "single" pollen, however, might even germinate and yet be strictly non-functional because of weak growth; and, as is shown below, the singles are actually inferior to the doubles in vigor. Selective partial sterility seems to be a rather common phenomenon, and it very probably occurs here.

The only other recourse seems to be the hypothesis of somatic segregation mentioned above, and somatic segregation, except as a rare accident of abnormal cell-division, has no decisive evidence in its favor[5] and an overwhelming convergence of probabilities against it. Belling (1915) calls attention to decisive evidence against

[5] Bateson's (1914, p. 292) positiveness in its favor seems to depend on just such cases as that here in question.

it in five genera representing as many distinct orders. Bateson himself (1909, chap. 9) reports a fact which seems to exclude it in the sweet pea, *although his reduplication hypothesis*[6] (Bateson and Punnett, 1911) *would require it there if anywhere.*

This phenomenon is one to which East (1915, p. 87) has recently referred, the "zygotic" nature of certain pollen-grain characters. In the sweet pea, for instance, F_1 hybrids between certain races with long (dominant) and round pollen have the pollen *all long,* although segregation, on any hypothesis, must have already occurred before the shaping of the pollen-grains. If segregation takes place as a result of chromosome-reduction, in the formation of the spore-tetrads, it is not strange that the cytoplasm of the young pollen-grain still retains the impress of the diploid maternal set of chromosomes, so that the pollen-grains give no evidence in their shape of the segregation that has just taken place. On the other hand, if segregation takes place early enough to permit of extensive "reduplications" of the cells carrying certain combinations of factors, it is very strange that the cytoplasm of the pollen-grain should be essentially maternal in nature. Especially does this evidence negative any hypothesis of cytoplasmic segregation—and if segregation is nuclear, surely we have reasons enough for connecting it with the reduction of the chromosomes.

It is due to Goldschmidt's hypothesis to note that a factor "completely coupled" with S, completely lethal for pollen and only slightly so for the embryo-sac, would explain the peculiarities of the case in *Matthiola,* both the non-functioning of the S-carrying pollen and the excess of doubles over 50 per cent. This is an amplification of his suggestion, in a passing reference (1913, p. 81), of a

[6] Bateson and Punnett explain linkage of genetic factors by means of the hypothesis of somatic segregation. They assume that a period of cell-division intervenes between the segregation of Mendelian factors and the formation of the germ-cells, and that the cells bearing certain sets of factors divide more often than the rest. This would result in making some classes of germ-cells more numerous than others.

possible "further distinct hereditary factor"; I merely omit the sex-factor, and suppose the other factor to be lethal in itself. He evidently does not notice that this sort of factor might well explain more than the sterility of the pollen. It amounts to the same thing, however, to suppose the double-thrower S (or S_1) to be itself the lethal factor. The introduction of sex-factors seems entirely unnecessary here, and the supposed lethal elimination of an F factor can not be general in hermaphroditic plants, since it would involve the universal occurrence of sterile microspores or pollen.

Our case appears to be merely one of a hybrid showing selective sterility of pollen-grains, a sterility due to the S factor or to a lethal factor linked with S. Further, if there is also a slight tendency to selective elimination of S-carrying eggs, we have a simple and direct explanation of the excess of doubles over the expected 50 per cent. Or, if the s-carrying eggs are more often fertilized, the excess of doubles is explained. Once more, selective elimination of single (Ss) embryos might produce the same result.

There are several facts which are extremely suggestive in relation to all these possible forms of selective elimination. First, it is known that, in a double-throwing race, the doubles are longer-lived than the singles in the seed stage; Miss Saunders (1911, p. 362) has definitely confirmed the common belief that the proportion of doubles tends to increase with the age of the seed. Second, Miss Saunders (1911, p. 364) has obtained a higher proportion of doubles from seed of lower viability, even with fresh seed. Third, some seed-growers (deVries, 1906, p. 335) regularly "starve" the seed-bearing plants, in the belief that they thus increase the percentage of doubles among the progeny. Fourth, the writer (Frost, 1911) has found, with one variety, that inhibition of flowering by high temperature is much more marked with singles than with doubles; in field cultures, in many cases, hot weather greatly delayed or entirely prevented flowering, the

difference there being very much greater than that shown in the tables in the paper cited.[7] Fifth, in the cultures just mentioned the doubles had larger leaves than the singles and evidently were decidedly larger as young plants. It seems that the double form (ss) is superior to the heterozygous single (Ss) of this double-throwing race in general vegetative vigor, and a similar difference may exist between s and S gametes; *on these facts probably depend the peculiarities of the observed ratio.*

In order to make the case of doubleness in *Matthiola* as clear as possible, let us consider a brief summary of the formulations that have been proposed. There are two essential points to be explained, namely: (1) the fact that the singleness factor or set of factors of the double-throwing races can not be carried by functional pollen, although the corresponding factor or factor-group of the pure single races so far tested is normal in relation to pollen, even in single-double hybrids; (2) the fact that the double-throwing races show a small but fairly constant excess of doubles over 50 per cent.

Miss Saunders gives a formally adequate but rather complex factorial hypothesis for (2). She leaves (1), however, essentially unexplained; she evidently relegates it to the realm of somatic segregation, and in any case makes no suggestion as to the real cause of the *uniform* elimination of *singleness.*

Goldschmidt, on the other hand, gives for (1) a hypothesis of selective sterility which is adequate, though of obviously unnecessary complexity, but fails with (2) about as completely as Miss Saunders does with (1).

It is here maintained that an extension of the general idea of selective elimination or viability, in any one of the several forms consistent with the evidence, complies with all the requirements, adequately explaining both (1) and (2). It might seem, at first thought, that the assumption of a difference between S and S_1, or of the exist-

[7] This evidence is to be published mainly in my forthcoming paper on "Mutation in *Matthiola.*"

ence of a distinct linked lethal factor, makes this scheme as complex as that of Miss Saunders; this is not the case, however, since Miss Saunders's scheme simply omits any attempt at real explanation of the peculiarity of the double-thrower pollen; her formulation imperatively requires the addition of the hypothesis of selective viability, or of some definite equivalent for it.[8]

The real puzzle of the case lies in the fact that the double-throwers plainly differ from the pure singles so far tested in at least two respects—heterozygosity for singleness (ability to form sporophylls) and the association of some peculiarity with the remaining singleness. This, however, is essentially a problem of the *origin* of the double-throwing races, and is, in any case, nowhere simpler than with the hypothesis here suggested. Miss Saunders's scheme really implies four factorial or linkage differences between pure singles and double-throwing singles, and for certain cases six such differences, in place of the two or three required by the hypothesis here favored. That is, the double-thrower is supposed to differ from the pure single in the following points: (1) that it is heterozygous for two complementary factors (X and Y) for which the pure single is pure, and in some cases also for a second set of such factors (X′ and Y′); (2) that its "singleness" can not be carried by functional pollen; (3) that X and Y are partially instead of completely linked. It is here proposed to drop half the factors of (1), and this makes (3) superfluous.

[8] She supposes (Saunders, 1911, p. 334) that X and Y are completely linked in the pure singles, but only partially so in the double-throwers; this explains why, with self-pollination, the latter give 50 + per cent. of doubles rather than 50 per cent., *but not why they approximate 50 per cent. instead of 25 per cent.* It would seem, however, that the Ss hybrid between pure single (SS) and double-thrower (S_1s) may usually give double progeny approximating, not the 25 per cent. assumed, but a *slightly lower ratio.* A possible general slight deficiency of doubles in this cross is not provided for in Miss Saunders's hypothesis, complete linkage of X and Y explaining why there is not an *excess* of doubles; whether the viability-hypothesis is adequate depends on the general viability-relations of the S factor (as distinguished from S_1), which quite possibly is even superior to s in respect to vigor.

It must be admitted, however, that (3) is not in itself improbable if (1) is true, in view of Miss Saunders's evidence. A similar difference between races, with respect to linkage, occurs with "cream" flower-color, which is partially linked with doubleness in the sulfur-white races, but completely linked in the pure-cream races. The essential difference of the viability-hypothesis, as here presented, relates to (1); the demonstrated lower viability of the singles, evidently the basis of (2), makes possible the simplification of (1).

If we accept this viability-hypothesis, there seem to be two general possibilities as to the origin of the double-throwing races. One is that the mutation by which Ss (double-throwing) races arise from SS (pure single) races involves a simultaneous or consequent alteration in the remaining S factor (or the production of a lethal factor completely linked with S), by which the presence of S becomes incompatible with pollen-formation. Second, it may be that the particular race or races in which our double-throwing forms originated had an S factor originally different from that of the pure single races which have been used in crossing with double-throwers—that is to say, an S factor originally incompatible with the formation of good pollen in an Ss plant—or else that they originally possessed the lethal factor suggested. If the second supposition is correct, such pure single races may be found,—races which in crossing with double-throwers *never give the* F_2 *ratio 3 singles:1 double, but only approximately 1 single:1 double.*

With *Petunia,* if we ignore the new seed-producing double (Francis, 1913), which has a distinct type of flower, the general case would seem to be similarly simple. Here, as is well known, the doubles are produced only when singles are pollinated by doubles, the ordinary doubles having stamens but not pistils, or, at most, non-functional rudiments of pistils. In this case the doubleness factor (D) is plainly dominant, and is perhaps to be considered an inhibitor; the single, then, is

dd, and the double Dd, cross-pollination giving 1dd:1Dd. There is usually (Saunders, 1910) an excess of *singles;* here, as in *Matthiola,* the heterozygous form is the one deficient in numbers, and it is also the one which appears inferior in vegetative vigor.[9] Probably the deviation from the 1:1 ratio is due in *Petunia* to selective elimination of *doubleness.*

We have, then, in *Matthiola* and *Petunia,* hybrids evidently due, not to the crossing of widely different forms, but to mutation within the race,[10] and yet they are partially sterile, and perhaps even lacking in vegetative vigor because of their hybridity! In connection with the vigorous discussion of mutation now going on, it seems worth while to ask whether, in a case like that of *Œnothera,* hybridization is the cause of mutation or mutation one great cause of hybridity; apparently both views may be in part correct.

Miss Saunders favors a dihybrid scheme for *Petunia,* evidently supposing the difference here also to depend on two complementary factors, both necessary for singleness. Her assumption that singleness is dominant, as in *Matthiola,* seems absolutely untenable. In considering the last point, we may ignore the dihybrid feature, since this evidently concerns only the deviation of the ratio from 50 per cent.

Her formulation, as thus simplified, makes the singles Ss and the doubles necessarily ss; the data then indicate that the functional single pollen is all S-carrying (the reverse of the case in *Matthiola*), since self-pollinated singles produce no doubles. Then, either the single eggs are S + s, and the double pollen s + s, or the single eggs are s + s, and the double pollen S + s. The latter assumption is obviously impossible, since it not only contradicts the assumption that singleness is dominant, but

9 Theodore Payne, a seedsman of Los Angeles, California, says in his 1914 seed-catalogue, "The weaker seedlings should be carefully saved, as these invariably produce the double flowers."

10 This is not to assume that some disturbance due to crossing of two single-flowering forms might not have led to the "mutation."

makes both singles and doubles heterozygous (Ss);[11] the former assumption, however, is also excluded, as Miss Saunders shows, by the fact that all singles tested produce some doubles when pollinated by doubles—that is, the expected class of pure singles (SS) does not occur. Evidently, as both Goldschmidt (1913) and Belling (1915) assume,[12] *doubleness* is dominant in *Petunia,* and selective viability probably completes the explanation.

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[11] In the dihybrid scheme, here, if linkage is to be invoked, as in *Matthiola,* to explain the deviation from 50 per cent. of doubles, both the singles and the doubles must carry *both factors*—since the singles possess both by hypothesis, and we are supposing the pollen of the doubles to show linkage! Further, doubles, not singles, would be expected to be in excess of 50 per cent. In fact, no self-consistent dihibrid scheme seems to be possible with *Petunia.*

[12] Goldschmidt's statement (1913, p. 84) seems to suggest that Miss Saunders herself made this change, especially as he refers to "*Journal of Genetics,* **1**, 1911"; apparently, however, he intends the original article, published in 1910, and I have failed to find any reference to the matter in the part of the volume published in 1911. The explanation given above was stated in a letter sent to her in May, 1914, but no reply has been received. Bateson (1913) considers singleness to be dominant, admitting the necessary conclusion that all cultivated singles appear to be heterozygous. How this universal heterozygosity could be maintained in self-pollination of the singles (S + s eggs × S pollen, since the opposite assumption is untenable), he does not explain.

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THE COAL MEASURES AMPHIBIA AND THE CROSSOPTERYGIA

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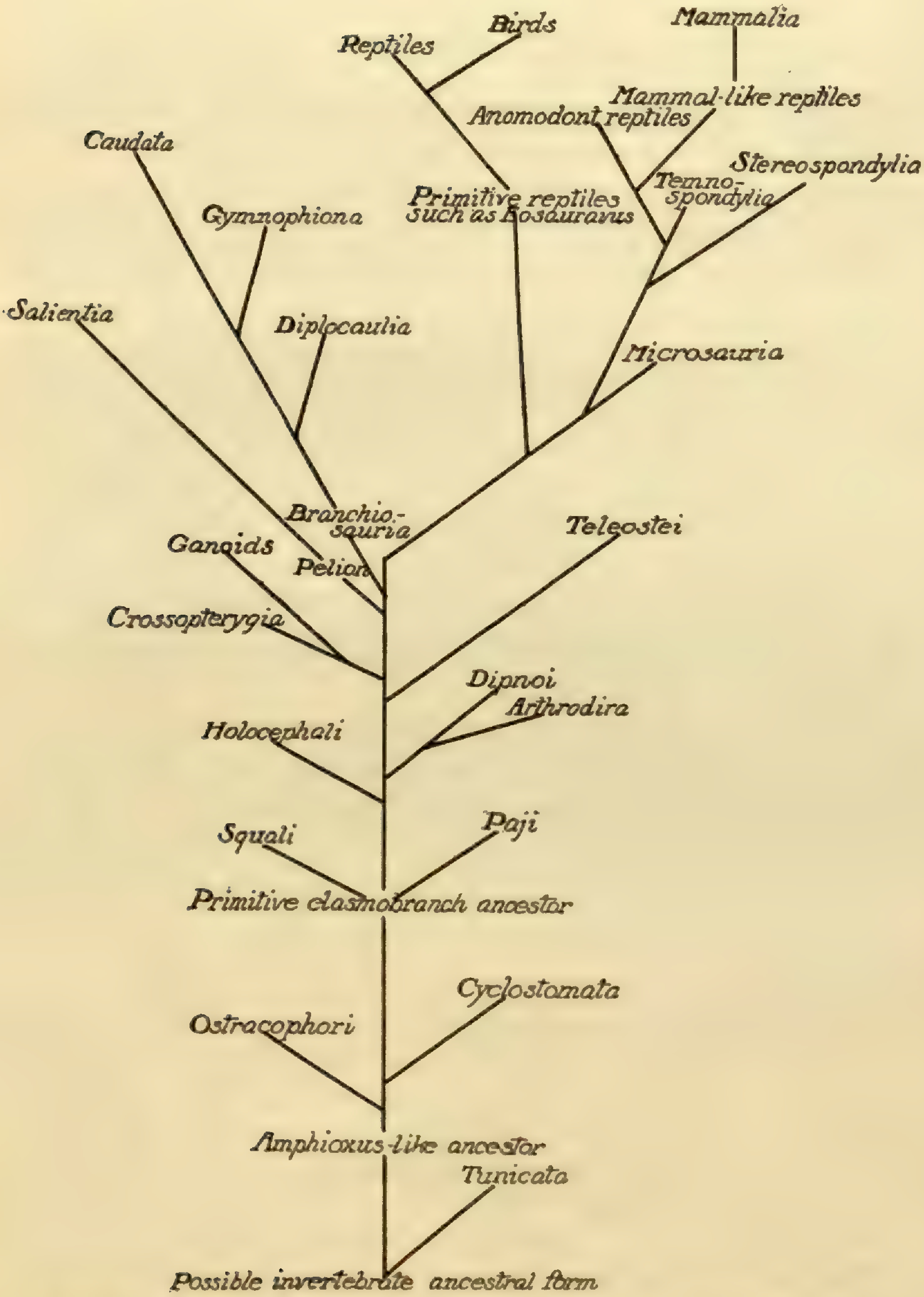
It has been assumed for many years that the crossopterygian ganoids are more nearly in the direct line of descent of the amphibia than any other known group of fishes. Recent work along this line adds considerable evidence to support this assumption and it is fast becoming accepted as practically proven that such was the line of descent of this group of vertebrates. Watson (1), Broom (2), Gregory (3–4), Pollard (5), Klaatsch (6), Budgett (7), the writer (8), and others have added to our knowledge of this relationship, which is based on the structure of the skull, the limbs and the mandible, so far as these anatomical features are known. Our knowledge of the osteology of neither group is satisfactory and it is to be hoped that additional material will do much toward a solution of this problem.

It is our purpose here to state in a brief manner what the Amphibia from the Coal Measures add toward the solution of the problem of the derivation of the amphibians from the crossopterygians.

The fish characters of the larval stages of the Amphibia have been often cited as evidence of this relationship. Budgett (7) says:

> It has been admitted by the most competent paleontologists that the structure of the dermal bones of the head and shoulder girdle of *Polypterus* is so like that of certain *Stegocephali,* that it must be regarded as more than a mere resemblance while there are many points in the development of the skeleton that distinctly approach the condition of the Amphibia. The only possible interpretation of these facts appears to me to be that the living crossopterygians form a central group among recent forms, having some characters in common with most of the great groups.

The writer (8) has called attention to the similarity of arrangement of the lateral line canals in Amphibia and Crossopterygia and has attempted a correlation of the cranial elements. In that essay it is stated:



A phylogenetic scheme illustrating in a tentative way the possible relationships of the Coal Measures Amphibia to the other Chordata. The Crossopterygia may have had more immediate ancestral relations than the diagram idicates.

The following elements of the stegocephalan cranium are homologous with the same elements in (the crossopterygian) fishes: premaxillæ, maxillæ, nasals, frontals, prefontals, parietals, squamosals, and postfrontals. The epiotics and supraoccipitals of the Stegocephala are homologous with the supratemporal elements of fishes. The quadratojugal is homologous with the subopercular of fishes. The supratemporal is homologous with the preoperculum (8).

Wilder has commented on the close relationship of these two groups and even goes so far as to say: "that terrestrial vertebrates were originally derived from a single form, perhaps a single species" (*i. e.*, of the Crossopterygia) (9). It will be interesting in this connection to give a brief résumé of the geological history of the two groups of vertebrates. The history of the Amphibia is briefly this:

Devonian: *Thinopus antiquus* Marsh, footprint from Pennsylvania.

Mississippian: Footprints from eastern North America.

Pennsylvanian: Five orders of Amphibians represented by hundreds of more or less complete skeletons from Europe and North America.

Permian: Four orders of Amphibia, known from an abundance of material from Europe, Africa, Asia, and North America.

Triassic: Two orders of Amphibia, the species of which may be compared very favorably with modern species; North America and Europe.

Comanchean: None known in North America.

Cretaceous: Caudata known from imperfect fragments, North America.

Eocene to Recent: Frogs and salamanders as in modern times.

Recent: Salientia, Caudata, *Gymnophiona.*

The geological history of the Crossopterygia is briefly summarized by Huxley[1] in the following:

The group of Crossopterygidæ as thus established appears to me to have many remarkable and interesting zoological and paleontological relations. Of the six families which compose it, four are not only Paleozoic, but are, some exclusively and all chiefly, confined to rocks of

[1] Scientific Memoirs, II, p. 445.

the Devonian age,—an epoch in which, so far as our present knowledge goes, no fish belonging to the suborders Amiadæ or Lepidosteidæ (unless *Cheirolepis* is one of the latter) makes its appearance. Rapidly diminishing in numbers the Crossopterygidæ seem to have had several representatives during the Carboniferous epoch, but after this period . . . they are continued through the Mesozoic age only by a thin, though continuous line of Cœlacanthini, and terminate, at the present day, in the two or three known species of the single genus *Polypterus,* now recognized under two genera: *Polypterus* and *Erpetoichthys* (*Calamoichthys*).

Such, in brief, is the geological history of this peculiar order of fishes. At the time of the publication of Huxley's essay the fact of the geological distribution of the group was of the greatest interest, since it proved the transition of a vertebrate group from the Paleozoic to later times in practically unchanged form.

It will thus be seen that in the Devonian we have representatives of these two groups; one of which has been supposed to have given origin to the other. The oldest known Crossopterygian coexisted, in the Devonian, with well-developed Amphibia-like forms if we may trust the evidence of the single imprint of *Thinopus antiquus* Marsh from the Devonian of Pennsylvania. That the impression described by Professor Marsh is in reality a footprint no one, who consults his figures, can doubt. Its geological horizon is vouched for by the late Dr. Charles E. Beecher, whose interpretations of geological facts have never been impeached. So that we may say with perfect assurance that the *oldest known Crossopterygia existed side by side, geologically, with well-developed air-breathing, quadrupedal vertebrates.* Whether the latter were Amphibia or not is a matter which no one, in the light of our present knowledge, can decide. The inference, however, is that they were such.

In order that all the facts of the case in regard to the origin of the Amphibia may be given, the following definition of Crossopterygia is given. It is based on the first definition by Huxley and the definition contained in Zittel's "Paleontology" (ed. by Eastman).

Dorsal fins two, or if single multifid and very long; the pectoral and usually the ventral fins lobate; no branchiostegal rays, but two prin-

cipal with sometimes lateral and median jugular plates situated between the rami of the mandibles; caudal fin diphycercal or heterocercal; scales cycloid or rhomboid, smooth or sculptured.

These characters may be supplemented by the following: Notochord persistent or vertebræ slightly ossified, infraclavicle present. Teeth dendritic in a few forms.

The following list contains brief statements concerning the structures in these two groups which are usually regarded as of great taxonomic importance in all vertebrates.

4. Comparative Table of Structures in Amphibia and Crossopterygia

Amphibia

I. Typically quadrupedal tetra- and pentadactyl, aquatic or terrestrial vertebrates. Body usually provided with ventral armor of ossified, calcified or chondrified myocommata, present in modern species as strands of connective tissue. Body completely scaled in one species. Scales present in other species but incompletely known.

II. Teeth labyrinthine in some species.

III. Vertebræ always ossified, sometimes highly developed; assuming various forms. Notochord incompletely persistent as intercentral masses.

IV. Skull covered with dermal bones, which are at times variously sculptured and grooved by pits and canals.

V. Lateral line canals present as distinct impressions in the dermal elements.

VI. Parasphenoid largely developed.

VII. The following cranial elements correlated with those of the Crossopterygia: Supraoccipital, epiotic, parietal, frontal, prefrontal, nasal, premaxilla, maxilla,

Crossopterygia

Aquatic, fring-fined, completely scaled, fishes with dorsal, ventral and caudal fins.

Dentine of the teeth dendritic in a few forms.

Vertebræ usually unossified or incompletely. Notochord largely persistent and but slightly constricted.

Skull covered by dermal bones which are seldom sculptured although possessing a similar type of lateral line system contained within the skin.

Lateral line canals not always impressions in bones, but similar in arrangement to the Amphibia.

Parasphenoid largely developed.

The following elements absent in Amphibia but present in the Crossopterygia: Supratemporal ossicles, ethmoid, hyomandibular, jugular plates, opercular apparatus consisting of operculum and suboperculum.

Pineal opening present in at least one genus.

Air bladder present, with at times calcified walls.

Pelvic girdle composed of rhomboid plate of two parts which may correspond to the ilia.

squamosal, quadrate, pterygoid, palantine, supratemporal, parasphenoid, postorbital. Ethmoid present in Gymnophiona.

VIII. Pineal opening present in most extinct species.

X. No air bladder. Lung anlagen on ventral surface of pharynx.

XI. Pelvic girdle composed of osseous ilium, ischium and cartilaginous or osseous pubis.

XII. Arm consisting of humerus, radius, ulna, carpals (sometimes cartilaginous), metacarpals and phalanges.

XIII. Leg composed of femur, tibia, fibula, tarsus (sometimes cartilaginous), metatarsus and phalanges.

XIV. Ribs single, long or short, heavy or slender, inter- or intracentral.

XV. Form of body not fish-like.

XVI. Mesonephros functional in adult.

XVII. External gills slender and thread-like in the young of all living and some of the ancient forms.

Pectoral fin composed of pro-, meta—, and mesopterygium, actinosts and fin rays. Broom (2) has correlated the elements in the arm of *Sauripteris taylori* with those of the amphibian arm down to the actinosts, which he regards as carpals. The fin rays would represent phalanges.

Pelvic fin composed of basiost which represents the femur, and meta- and mesoptergyia and fin rays.

Ribs double; dorsal, arising independently of transverse processes; ventral one an exogenous process of vertebra, which in the tail becomes the hæmal arches by fusion of the tips.

Form of body always typically fish-like.

Mesonephros functional in adult.

External gills of young, slender, thread-like, recalling those of larval salamander.

It will be seen from the above list of comparative structures that there are a variety of instances in which the two groups approach each other. It is, however, to be clearly kept in mind that the oldest known Amphibia, as indicated by our present knowledge of these forms, are more like the modern forms of Amphibia than they are like the ancient types of fishes from which they supposedly have been derived. In other words, a more complete knowledge of the Coal Measures Amphibia has not served to simplify our ideas of amphibian descent in the least, but rather to confuse them.

All of the early Amphibia have well-developed ambulatory or natatory limbs and none of them are fish-like in

external form. Many of the representatives of the Amphibia in the Coal Measures of North America are highly specialized and adapted for a variety of modes of life. One of the most significant factors in the derivation of the early land vertebrates from the fishes is the question of the origin of limbs from fins, on which much has been written from a theoretical standpoint, but nothing has been seen in the nature of material supporting and defining the details in the process of evolution. It must be remembered, however, that the Coal Measure forms are Amphibia in a high stage of development and when new discoveries show us the anatomy of the forms from the Mississippian, Devonian and possibly the Silurian, then we shall be in better shape to discuss the question of the origin of tetra- and pentadactyl limbs.

The evolutional status of the Coal Measures Amphibia may be briefly stated. They were an assemblage of forms, highly developed and highly specialized, with few primitive characters which would tend to ally them directly with any known group of more primitive vertebrates. We should expect to find among these Paleozoic Amphibia some evidence of a transitional type of limb (11) structure between that of *Eusthenopteron* (12), or allied Crossopterygian, on the one hand, and the pentadactyl terrestrial vertebrate, on the other. But such evidence is not forthcoming among the material at present available. Evolutional forces had brought about a wide diversion of faunas in the Coal Measures, so that the species are easily separable into distinct geographic groups, which are more distinct than are the species of Amphibia inhabiting the same regions to-day.

The living Amphibia are much more commonly identical in eastern Ohio and northern Illinois than were the species of the same order during the Coal Measures. Species of *Necturus, Amblystoma, Rana* and *Bufo* are not widely different in the two localities referred to; yet the species of Amphibia during the Coal Measures at Mazon Creek, Illinois, and Linton, Ohio, are widely distinct. They are, in fact, more widely distinct than the species of

modern Amphibia existing in New York and in California; for in these states we find modern genera in common. This wide diversion of structure between the faunas of Linton and Mazon Creek is not due to difference in age, since it is almost assured that they were nearly contemporaneous geologically.

The high degree of specialization attained by various members of the Coal Measures Amphibia is remarkable. They had become adapted to nearly every condition of vertebrate existence, which animals of later times have adopted. There were strictly aquatic, fossorial, terrestrial, climbing, crawling, worm-like, snake-like, lizard-like, crocodile-like, all living contemporaneously. The absence of fish-like forms is noteworthy.

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6. Klaatsch. Hermann. 1896. Die Brustflosse der Crossoptyeygiaer. *Festschr. für Gegenbaur*, Bd. I, pp. 259–391, Taf. I–IV.
7. Budgett, J. S. 1902. On the Structure of the Larval Polypterus. *Trans. Zool. Soc.*, London, XVI, Pt. VII, October; Works, pp. 154–176, pls. X–XIII, 1907.
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9. Wilder, H. H. 1909. History of the Human Body. New York, Henry Holt and Co. Amphibia, pp. 541–2.
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SHORTER ARTICLES AND DISCUSSION

AN ANTICIPATORY MUTATIONIST

Whenever any new view gains acceptance it is usually found to have been partially anticipated in the writings of various authors. The mutation theory is no exception to this rule, and the purpose of this note is to direct wider attention to the anticipation of mutationist views by Thomas Meehan. While it is known to some that Meehan held such views, it is not, I think, generally realized how consistently and persistently he advocated them throughout the course of his life.

Thomas Meehan was born near London in 1826, was trained as a gardener at Kew and afterwards came to America. He settled in Philadelphia as a horticulturist, became a prolific writer for agricultural and horticultural journals and finally, in 1891, established *Meehan's Monthly,* a journal devoted to gardening. He traveled as far as the Rocky Mountains and Alaska, was appointed state botanist for Pennsylvania, and was in 1875 elected a fellow of the American Association for the Advancement of Science. His publications included "Native Flowers and Ferns of the United States," in four volumes, and the current of his work continued until after his death in November, 1901.

But the phases of his active life which I wish to emphasize here were (1) his keenness and accuracy as an observer, and (2) his constant advocacy of discontinuity in the variations of species on the basis of his own observations, at a time when such views were by no means popular. Meehan was particularly active in the Philadelphia Academy of Science, and he contributed in all no less than 257 papers and notes to the *Proceedings* of that society between the years 1862 and 1901.

Although Meehan accepted evolution with his contemporaries and with Darwin, yet he never lost an opportunity to emphasize the probable significance of the wide variations which he frequently observed in nature, as opposed to the insensible changes which were believed to furnish the material for evolution. The character of his observations as well as the trend of his views, may be indicated by a few quotations from his writings.

In a paper entitled "Change by Gradual Modification not the Universal Law,"[1] he begins as follows:

> *Natura non facit saltum* has been accepted as a grand canon by most naturalists, and the evident absence of connecting links has been thought fatal to theories of evolution. My studies in plant life lead me to the belief that one form will spring from another essentially different, and without any gradual or insensible modifications uniting them.

He then describes a variation in *Halesia tetraptera*.[2] The new form had undergone a change in leaf shape and the veins were rugose. The flowers, instead of having a narrow tube at the base, were open, cup-shaped, the pistil wholly enclosed and not exserted. This form produced good seeds and if found wild would be considered a new species.[3] He then refers to a variation in *Yucca filamentosa*. One plant in hundreds threw out a more branching panicle which opened two weeks earlier. Its characters remained and were continued in the progeny. After citing other cases, Meehan says:

> Not only do strikingly distinct forms come suddenly into existence but once born they reproduce themselves from seed, and act in every respect as acknowledged species.

He states that a "weeping" variety of the peach came into existence "about 30 years ago," and also "ten years ago a deep blood-leaved variety appeared." The following quotations from the same paper will serve further to illustrate his views:

> In over a quarter of a century of experience among living plants, I have rarely known any striking form to have originated by gradual modification, but always by one great leap. The slight changes are generally in efforts backwards; as when we sow purple beech seed, some few are a trifle paler than their parents; there is little or no hesitation in the forward leap. . . .
>
> Forms are not only called into existence suddenly, widely different from their parents, and can reproduce themselves from seed, but they come into existence without seed agency, and the same or similar form in widely separated localities, and not all necessarily by seed from one individual.

Flowers of *Viola pedata* were sent to him from five localities in Pennsylvania, New York, Illinois and Indiana, having "the two upper petals a beautiful maroon color as in the pansy."

[1] *Proc. Amer. Assoc. Adv. Sci.*, 1874, B. 7–12, 1875.

[2] Now known as *Halesia carolina* L.

[3] This fuller account is taken from his later paper, "Variation in *Halesia*," *Proc. Phila. Acad.*, 1884, 32–33, Figs. 4, 1885.

Among the conclusions of this paper, which pretty well sums up his views, we find,

> Morphological changes in individual plants are by no means by gradual modification,

and

> New and widely distinct species may be suddenly evolved from preexisting forms without the intervention of connecting links.

A discussion followed in which Professor Morse, C. V. Riley, Professor Gill and Asa Gray took part, but although some agreed that there was no reason why marked changes and gradual modifications should not both play a part in evolution, yet the tendency was rather to look upon the former as sports which were of little evolutionary significance. Meehan afterwards referred to this paper as showing that:

> New forms "jumped" into existence, and frequently these new forms were diverse from each other, under precisely the same "environment" as far as human knowledge had yet reached, as had been the surrounding circumstances of the parent form.

Quotations of a few of Meehan's other papers, with notes upon them, will serve to show the range of his ideas and the accuracy of his observations.

"On the Agency of Insects in Obstructing Evolution," *Proc. Phila. Acad.*, 1872, 235–37, 1872.

"On Rapid Changes in the History of Species." *Proc. Phila. Acad.*, 1884, 142–43, 1885.

"Persistence in Variations Suddenly Introduced," *Proc. Phila. Acad.*, 1885, 116, 1886.

> We see that identical forms may appear simultaneously in localities widely separated; and, the circles meeting, cover a district in a comparatively short time.

"Spicate Inflorescence in *Cypripedium insigne*," *Proc. Phila. Acad.*, 1885, 30–32, 1886.

> Such a belief [in jumps] would tend materially to remove difficulties in the way of theories of evolution, that now prevented a full acceptance thereof.

"On a White-seeded Variety of the Honey Locust," *Proc. Phila. Acad.*, 1885, 404, 1886.

In this paper he describes a tree of *Gleditschia triacanthos* growing near Germantown, Pa., which had seeds white instead of dark olive-brown. They also differed in shape, being nearly

orbicular, instead of narrowly ovate, and twice as long as broad. He remarks in this paper:

> When variations occur it is difficult for some to believe that cross-fertilization, a return to some characteristic of an ancient parent, or some accident of climate or soil had not [been] an agency in the change.

This type of difficulty is still formidable in the minds of some. In the case he describes such explanations are excluded as inapplicable.

"On Parallelism in Distinct Lines of Evolution," *Proc. Phila. Acad.*, 1886, 294–95, 1887.

Meehan refers[4] to a paper given by him at the Troy meeting of the American Association (1870), "On the Introduction of Species by Sudden Leaps." But there is no such paper in the report, although he gave three other papers dealing respectively with fasciation, pollination by insects, and the influence of nutrition on sex. His last paper, published posthumously, in *Proc. Phila. Acad.*, 54, 33–36 (1902), is in two parts, dealing with "The Bartram Oak, in Connection with Variation and Hybridism" and "Observations on the Flowering of *Lobelia cardinalis* and *Lobelia syphilitica*." He may well be described with justice and accuracy as an anticipator of the mutation theory, not on theoretical grounds but on the basis of his own keen observations.

Meehan also contributed to the earlier volumes of the American Naturalist, the *Botanical Gazette*, *Torrey Bulletin* and other journals during his active life. One of these[5] characteristically sets forth his mutationist views.

R. Ruggles Gates

[4] *Proc. Phila. Acad.*, 1885, 30.

[5] On the Relation Between Insects and the Forms and Character of Flowers," *Bot. Gazette*, 16, 176–77, 1891.

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VARIABILITY AND AMPHIMIXIS

PROFESSOR L. B. WALTON

KENYON COLLEGE

A COMPARATIVE STUDY OF THE VARIABILITY IN ZYGOSPORES OF *Spirogyra inflata* (VAUCH.) FORMED BY LATERAL (CLOSE BREEDING) AND BY SCALARIFORM (CROSS BREEDING) CONJUGATION, AND ITS BEARING ON THE THEORY OF AMPHIMIXIS AND CORRELATED PROBLEMS

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I. Preliminary Outline

1. *Introduction*

Comparative studies along statistical lines of the results produced by cross breeding and close breeding afford data of value bearing on the problem of evolution as well as the subsidiary problem of the origin of amphimixis. It has long been assumed (Weismann, '76) that sex existed primarily to increase variability and with the further assumption that the variations thus produced were heritable and accumulated, the differentiation of organisms was logically explained. As a corollary to such a conclusion the belief has long been prevalent that the offspring of organisms produced by cross breeding were as a group more variable than those produced by close breeding, an idea which gained further acceptance in connection with the investigations of Castle ('06), Jennings ('08, '09, '12, '13) and others interested in problems of genetics. That there was excellent evidence for exactly an opposite view and that an analysis of the results presented by the investigators mentioned above did not bear out the conclusion that variability was increased by cross breeding has been pointed out by the writer (Walton, '08, '12, '14) in some earlier papers.

The importance of arriving at a correct conclusion concerning the part played by hybridization and cross breeding in evolution can not be overestimated. If units are merely redistributed and form characters resulting in no actual evolutionary progress, work along Mendelian lines tends rather to obscure the facts of value toward solving the problem of the origin of species as well as that of evolutionary control in animal and plant breeding. It is therefore well to obtain data from as many sources as possible bearing on the question.

Among the species of *Spirogyra,* a group of algæ belonging to the class Conjugatæ, there are several which reproduce both by lateral conjugation (Fig. 1, *A*) where

the adjacent cells of a single filament unite to form the zygospore, itself a young individual, and at the same time by scalariform conjugation (Fig. 1, *B*) where the cells of two distinct filaments unite to form the zygospore. Thus

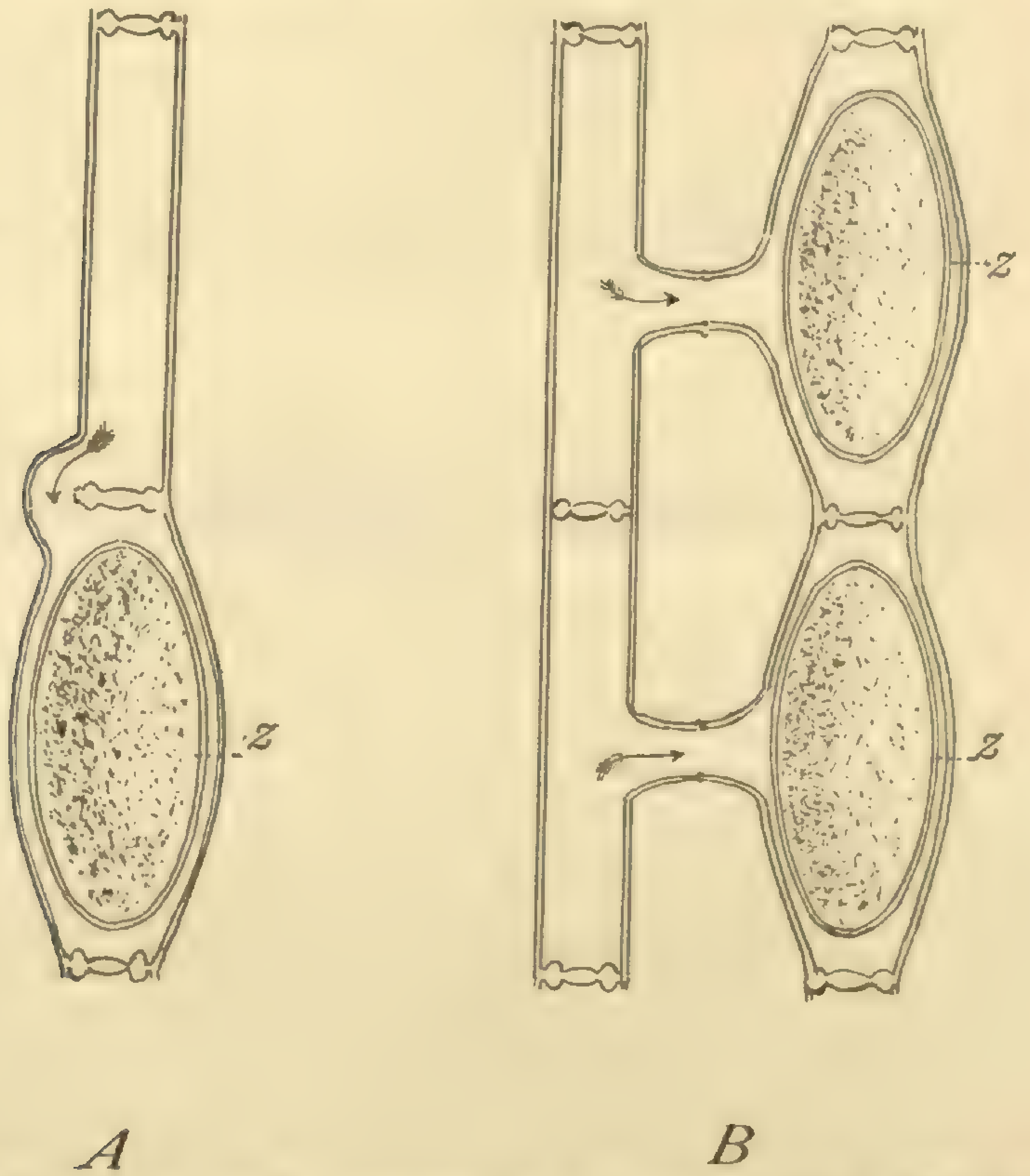

FIG. 1. FORMATION OF ZYGOSPORES IN *Spirogyra inflata* (Vauch.) by lateral conjugation (*A*) close bred from the same filament, and by scalariform conjugation (*B*) cross bred from two distinct filaments. *z* = zygospore.

there is an example of a population producing under the same environment two groups of individuals, one by close breeding (lateral conjugation) and the other by cross breeding (scalariform conjugation), and a comparison of the variability by statistical methods should afford evidence toward the solution of the problem presented where the offspring have arisen from a common ancestor as indicated in the material studied.

2. *Historical*

Much has been published concerning hybridization, cross and close breeding, amphimixis and parthenogenesis, all of which are distinguishable from one another merely by degree, nevertheless so far as the subject under

discussion is concerned, the conclusions in general have largely been assumptions based on little or no evidence.

It was Weismann ('76) who was evidently the first to definitely express the importance of sex in producing variations, an idea to which he consistently held in his subsequent papers, while Nägeli ('84), Strasburger ('84), Hatscheck ('87), Haycraft ('95), etc., believed likewise on theoretical grounds that variability was reduced by amphimixis.

The first paper presenting tangible evidence upon the subject was that of Warren ('99) who found that parthenogenetically produced *Daphnia magna* were slightly more variable as measured by the "Standard Deviation" which had a value of 2.95, than the mothers whose "Standard Deviation" was 2.22. The small number utilized, 96 in the first instance and 23 in the second instance, together with the fact that the mothers represented a selected class, only those *Daphnia* producing young being included, did not allow placing much reliance in the results. Warren ('02) compared 60 parental aphids (*Hyalopterus trirhodus*) and their 368 offspring as well as a series from 30 aphid grandparents and their 291 grandchildren. The variability was found in a comparison of grandparents and grandchildren (parthenogenetic) to have slightly decreased in respect to frontal breadth and considerably increased in respect to length of right antenna, but again objections similar to those in the preceding paper render the conclusion of little value, as Warren himself observed.

Casteel and Phillips ('03) measured drones and workers of *Apis mellifica,* the honey bee, selecting individuals at random from different colonies, and tabulating classes and frequencies without, however, a further application of biometrical methods. The "range of variability" was found to be greater in the drones than in the workers. Lutz ('04) criticized the methods utilized in the paper, nevertheless variation as measured by the standard devia-

tion upon calculation by Wright, Lee and Pearson ('07) was found greater in the drones by a difference ranging from 0.22 to 2.63 in respect to all five characters studied in the single group of 50 Italian workers and 50 drones of real value for comparative purposes.

Kellogg ('06), in a preliminary paper dealing with drones and workers of bees and also with female aphids, concluded that not only was there no evidence that amphimixis produced increased variability, but that it was an unnecessary factor in the production of Darwinian variation. The results were summarized as follows:

(*a*) In all but one of the characteristics studied, the amount of variation both quantitative and qualitative, is markedly larger among the drone bees than among the workers, and in the one exceptional characteristic it is no less; (*b*) no more variation in wing characters is apparent among drones or workers that have not been exposed in imaginal condition to the rigors of personal selection than exists among bees, drones or workers, that have been so exposed; (*c*) the variation in wing characters in drone bees reared in worker cells is no greater than that among individuals reared among drone cells; (*d*) the variation among drones hatched from worker laid eggs is markedly larger than that among drones hatched from queen laid eggs. . . ."

Eleven "lots" were studied with a small number (No. 3, 48; No. 7, 54; No. 8, 75; No. 9, 26; No. 11, 60) in many of the "lots." Even though the probable errors would have been large and while the material was heterogeneous, the facts brought out are of extreme interest, particularly when considered with the results obtained by Casteel and Phillips ('03).

Wright, Lee and Pearson ('07) made a comparative biometrical study of 129 queens, 130 drones, and 129 workers taken from a nest of the common wasp *Vespa vulgaris* in Charterhouse, England. In connection with the wing dimensions, the coefficient of variation was found to be greatest in the worker, less in the drone, and least in the queen, differing from the bee as noted above where drones were more variable than workers. The conclusion here of interest was:

There is no evidence in favor of parthenogenesis resulting in a smaller variability than sexual reproduction, for if the workers be more, the queens are less, variable than the drones.

It was suggested by the writers that the large variabilities of the workers might have resulted from subclasses among them due to differentiated functions or nurtures.

Castle, Carpenter, Clark, Mast and Barrows ('06) made observations on the variability and fertility of *Drosophila ampelophila* Loew, the small fruit fly, as modified by inbreeding and cross breeding. They found that "inbreeding does not affect the variability in number of teeth on the sex comb of the male, nor the variability in size." While the conclusion is not in accord with an earlier observation (p. 780) that variability would seem to have been increased by inbreeding so far as a comparison of the sixth inbred generation with the sixty-first generation, the small number utilized in the sixth generation (40 males in series *A*–6, *B*–6, *C*–6 each) was ground for the opinion that such a conclusion had little value in comparison with data pointing in the reverse direction. If however we calculate the coefficient of variation for the length of the tibia, an unfortunate omission on the part of the writers, it may be noted that the flies produced by inbreeding are decidedly more variable than those produced by cross breeding. Data for this conclusion are given in a subsequent part of the present paper.

Walton ('08) noted that the results of measuring zygospores of *Spirogyra* indicated that the close-bred individuals were more variable than the cross-bred individuals and furthermore that the data went far toward confirming the theory that sex existed for the purpose of limiting instead of augmenting variability.

Emerson ('10) found that crosses between races of plants (maize, squash, beans, gourds) differing in size and shape had the variability of the second (F_2) generation approximately twice as great as the variability of either parental form or of the first (F_1) generation. This he

explained on the basis of the segregation of size and shape characters. Similar results were obtained by East ('11) for maize and Hayes ('12) for tobacco.

Jennings ('11) extending and summarizing his breeding experiments on Paramecium concluded that

> The progeny of conjugants are more variable, in size and in certain other respects, than the progeny of the equivalent non-conjugants. Thus conjugation increases variation.

Later ('13) continuing his investigations he stated that conjugation increased the variability in the rate of reproduction. In a subsequent part of the present paper a somewhat critical review of the data and conclusions therein noted is presented.

3. *Material*

In obtaining material early one April for the laboratory work of a class in biology, the collection being made in a small pool resulting from the overflow of a rivulet, a peculiar species of *Spirogyra* was noticed in which both lateral and scalariform conjugation was taking place often in the same filament. It was at once suggestive that a comparison of the variability in the two groups of zygospores would present facts of interest in connection with the effect of close breeding and cross breeding on variability as well as affording evidence as to the theories of amphimixis.

The species was first determined as *Spirogyra quadrata* (Hass.) but subsequent examination indicated that it should be classified as *Spirogyra inflata* (Vauch.).

The material utilized for the measurements was all procured at one time from a restricted area one or two inches square on the surface of the pool and included only the one form of *Spirogyra,* that alone being present as a mass 3 or 4 inches in diameter. Inasmuch as both lateral and scalariform conjugation occasionally took place in the same filament (Fig. 2) a suggestion that two species were

represented can not be made for the filaments are alike in every characteristic. Of the 500 zygospores observed 45 per cent. were produced by lateral conjugation.

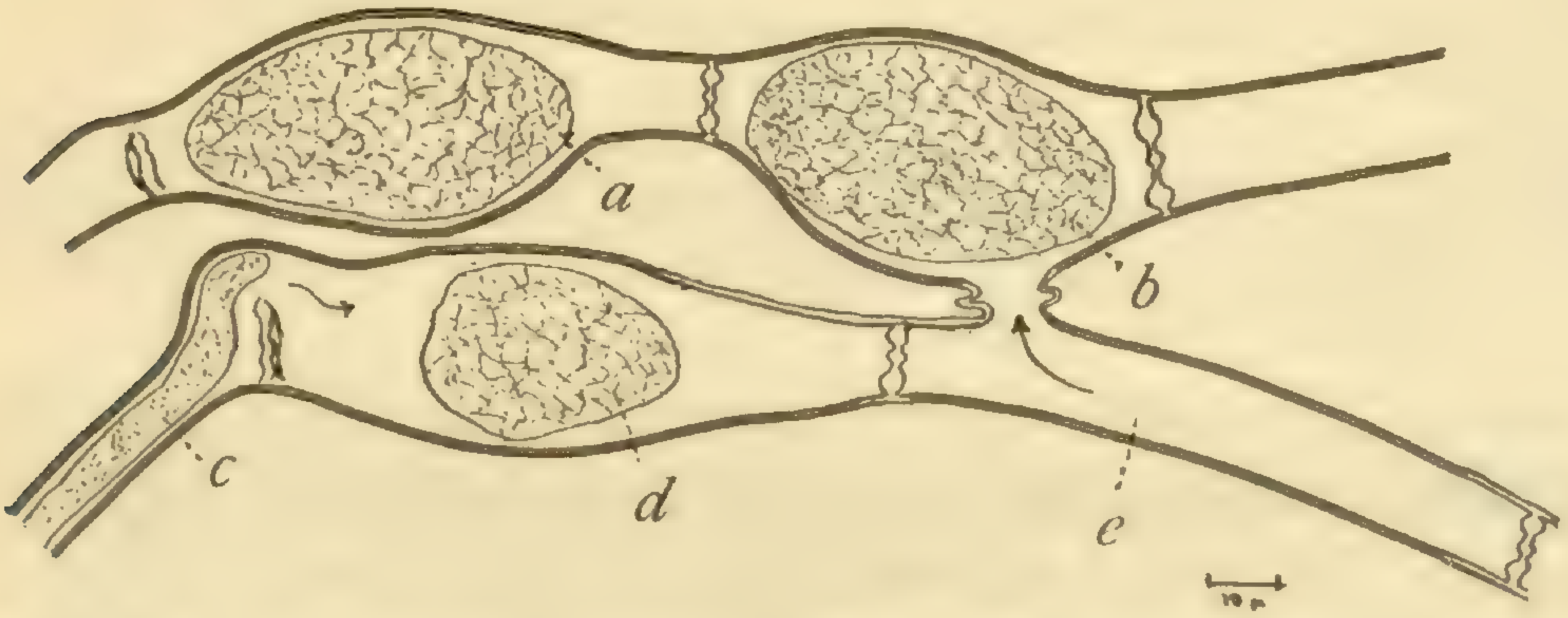

FIG. 2. *Spirogyra inflata* (Vauch.) ×800, with both scalariform and lateral conjugation in the same filament. (*a*) Zygospore formed by lateral conjugation. (*b*) Zygospore formed by scalariform conjugation. (*c*) Protoplasm of a "male" cell uniting with (*d*) the protoplasm of a "female" cell to form a zygospore by lateral conjugation. (*e*) Cell from which the protoplasm has passed to form the zygospore in (*b*). Obj. 1/12, Ocul. 2. Camera lucida drawing.

4. *Methods*

In considering the variability of large numbers of microscopic forms, rapid and accurate measurements are a necessity. Pearl and Dunbar ('03) in measuring *Arcella* used a camera lucida, marking the dimensions by means of a needle point, and reducing to microns. Pearl ('06) adopted a similar method for *Chilomonas,* using a magnification of 689.7. Pearl ('07) in measuring *Paramecium* used a 2/3-inch objective and a No. 1 ocular. By means of a camera lucida the points to be measured were projected on cards, marked, and measured with a vernier calipers to tenths of millimeters. Multiplying the measurements so obtained by the proper reduction factor found by calibrating with a stage micrometer, they were reduced to microns and recorded. Jennings ('11) at first measured *Paramecium* from a slide with an ocular micrometer. Later an Edinger drawing and projection apparatus was used, the projected images of the specimens on a slide in a flat drop of 25 per cent. glycerine, with-

out a cover glass which by pressure would have caused distortion, were enlarged to 500 diameters and measured with a milimeter ruler.

In the present study, the material was preserved in 2 per cent. formalin, the first series of measurements[1] being made April 2, while measurements of 358 were completed before May 16, and the remaining 42 finished Aug. 21 of the same year. Swelling of the zygospores did not occur to an appreciable extent, a possible error considered in a subsequent part of the paper. Using a B. and L. BB-6 microscope with a No. 1 ocular and a 1/12 oil immersion, a slide with a couple of drops of fluid containing the material was covered with a No. 2 coverglass, the superfluous liquid drawn off by means of a pipette, and the preparation placed on the mechanical stage. Beginning at the lower right-hand corner the slide was moved from left to right and each zygospore presented in the field in a uniformly horizontal condition, was measured. On reaching the left margin of the preparation, the slide was returned to the first position, moved sufficiently toward the observer so that a new path would be traversed, and the operation repeated. Thus the selection was at random and no zygospore measured twice. The dimensions were marked on note paper by means of a camera lucida at a magnification of 1,460 diameters, the two cross lines representing length (x) and diameter (y) having at the point of juncture an "*S*" or an "*L*" for scalariform or lateral conjugation. Only those zygospores having definitely formed membranes were considered.

In the reduction of data, so soon as the projections of the apparent dimensions were completed, the length of the lines x and y were measured with proportional dividers (Keuffel and Esser No. 441 special) adjusted at the ratio 1,460 to 1,000, thus giving a reading in tenths of microns. Accurate adjustment was made possible by means of a

[1] I am indebted to Dr. C. C. W. Judd, of Baltimore, Md., at that time a senior in Kenyon College, for work in part in obtaining the first series of measurements.

micrometer screw, on the basis of the equation for similar triangles;

1,460 mm.: 1,000 mm.= 160 mm.— x mm.: x mm.

where 160 represented the total length of the dividers, and x or 65.04 mm. the point of adjustment. Having checked the adjustment, it only became necessary to note the size of a given zygospore with the longer legs of the instrument, then by applying the shorter legs to a millimeter scale, to read the result. The various constants were then computed on the basis of the work of Pearson and of Elderton by means of a Brunsviga calculating machine. I am indebted to Dr. H. H. Mitchell of the University of Illinois for checking the mathematical data.

II. Consideration of Results

The direct results obtained by the statistical methods employed are here presented. These furnish the basis for the general discussion and conclusions which follow. The problems of biology relating to evolution need the application of statistical methods to studies in genetics. In no other way will it be possible to clearly demonstrate the relative efficiency of the different types of variation—fluctuation, amphimutation, cumulation, etc.—in originating and maintaining the diverse forms of life that exist. Similarly the importance or unimportance of small variations in animal and plant breeding may only thus be explained. The refinements of curve fitting are by no means necessary, nevertheless values are thus exhibited which are presentable in no other way.

1. Comparative Variability in Length of Zygospores

In the frequency distribution for lengths of the two groups of zygospores (Table I) the class range adopted was two microns as compared with a range of one micron in the distribution of diameters. The more extended as well as the more irregular distribution of lengths of the lateral zygospores when compared with the scalariform

zygospores is at once suggestive that the group thus close bred, is the more variable one. It is also of some interest to note that the empirical range of variation for the laterally formed zygospores,—with length from 49 m. to 83 m.,—is considerably greater than in the scalariform zygospores with lengths from 47 m. to 79 m. While this is not a measure of statistical variability, it undoubtedly has a genetic value.

TABLE I

LENGTH OF 400 ZYGOSPORES FROM *Spirogyra inflata* (VAUCH.), 200 PRODUCED BY LATERAL CONJUGATION AND 200 PRODUCED BY SCALARIFORM CONJUGATION, ARRANGED IN CLASSES ACCORDING TO FREQUENCIES. MAGNITUDES IN 1/10*m*

Length of zygospores in microns.

Lateral Conjugation			Scalariform Conjugation	
Class	Frequency		Frequency	
	Observed	Calculated	Observed	Calculated
38.0–39.9	1	0.21	0	0.
40.0–41.9	0	0.43	0	0.
42.0–43.9	1	0.84	0	0.
44.0–45.9	3	1.49	0	0.
46.0–47.9	2	2.56	3	1.17
48.0–49.9	0	4.18	3	3.14
50.0–51.9	4	6.44	6	6.76
52.0–53.9	16	9.34	16	12.65
24.0–55.9	10	12.71	20	19.82
56.0–57.9	26	16.16	25	26.21
58.0–59.9	11	19.17	25	29.40
60.0–61.9	19	21.15	26	28.41
62.0–63.9	22	21.67	25	24.13
64.0–65.9	20	21.18	18	18.30
66.0–67.9	21	18.10	11	12.66
68.0–69.9	13	14.72	7	8.12
70.0–71.9	16	11.08	6	4.90
72.0–73.9	5	7.72	6	2.83
74.0–75.9	1	4.98	1	1.57
76.0–77.9	5	2.99	1	.85
78.0–79.9	2	1.66	1	.45
80.0–81.9	1	.86	0	0.
82.0–83.9	1	.42	0	0.
Total........	200	200.06	200	201.04

The general constants for the variability in the length of the zygospore of the two groups are shown below (Table II). It may be noted that the mean (M.) or aver-

TABLE II

GENERAL CONSTANTS FOR VARIATION IN LENGTHS OF ZYGOSPORES BASED ON 200 FORMED BY LATERAL AND 200 FORMED BY SCALARIFORM CONJUGATION WITH A CLASS RANGE OF 2 MICRONS. STANDARD DEVIATION IN MICRONS

Constant		Lateral Conjugation (Close Bred)		Scalariform Conjugation (Cross Bred)	
Name	Symbol	Value	Prob. Error	Value	Prob. Error
Number	N.	200	....	200	....
Mean	M.	62.38	±0.1776	60.44	±0.1345
Mode	Mo.	62.5123	....	59.5170	....
Standard deviation	σ.	7.4460	±0.1304	5.7474	±0.1104
Coefficient of variation	CV.	11.9364	±0.0330	9.5093	±0.0330
Skewness	Sk.	−.0356	±0.0468	.1589	± .0464

age length of the zygospores produced by lateral conjugation exceeds the mean of the scalariform conjugants by 1.94 microns, while the probable error for the first constant is ±.1776 and for the second constant ±.1345. The difference is therefore a significant one so far as the present material is concerned.

It is in the comparison of the standard deviations (σ) and the coefficients of variation (C. V.) that the results of most interest appear, however. The former constant in lateral conjugation has a value of 1.6986 in excess of the same constant in scalariform conjugation, or relatively 29 per cent. This is more than thirteen times the probable error. In the coefficient of variation, an abstract number permitting comparison with similar constants in other organisms, the results indicate that the variability in lateral conjugation exceeds that occurring in scalariform conjugation by 2.4271 or relatively 26 per cent., a result corroborated by the distribution of the diameters. The probable errors are sufficiently small in comparison with the differences noted, that they may be considered negligible.

Skewness is negative in the curve for lateral conjugation, the mean being on the left side of the mode, but its value is less than the probable error. In the curve for scalariform conjugation skewness is positive with a value

slightly more than three times the probable error. Therefore the differences of the two constants appear to have no particular value so far as the present material is concerned.

The analytical constants (Table III) necessary for the

TABLE III

ANALYTICAL CONSTANTS FOR VARIATION IN LENGTH OF ZYGOSPORES FORMED BY LATERAL SCALARIFORM CONJUGATION.

Constant	Lateral Conjugation	Scalariform Conjugation
m_2	13.8606	8.2583
m_3	—3.9024	9.2989
m_4	606.8690	245.0223
B_1	.0057	.1535
B_1	.0756	.3918
B_2	3.1068	3.5928
k	.0219	.1654

fitting of the curves indicate that type IV curves may be used for each method of conjugation. In lateral conjugation the equation is

$$y = 10.842\left(1 + \frac{x^2}{29.2687^2}\right)^{-33.0942} \times e^{-9.5995 \tan^{-1}(x/29.2687)}$$

and in scalariform conjugation similarly the equation is

$$y = 5.0014\left(1 + \frac{x^2}{529}\right)^{-11.0945} \times e^{-8.9389 \tan^{-1}(x/11.50)}$$

while the frequency polygons and the fitted curves (Figs. 3, 4, and 5) illustrate the conditions diagramatically.

[2] The following formulæ as the basis of the probable errors, may be noted:

$$PE_\sigma = .67449\sqrt{\frac{M_4 - M_2^2}{4M_2}} \Big/ n, \quad PE_{\text{C. V.}} = \frac{.67449}{\sqrt{2n}}\left[1 + 2\left(\frac{v}{100}\right)^2\right]^{\frac{1}{2}},$$

$$PE_{\text{Sk.}} = .67449\sqrt{\frac{3}{2n}} \Big/ \sqrt{1 + 3(\text{Sk.})^2}.$$

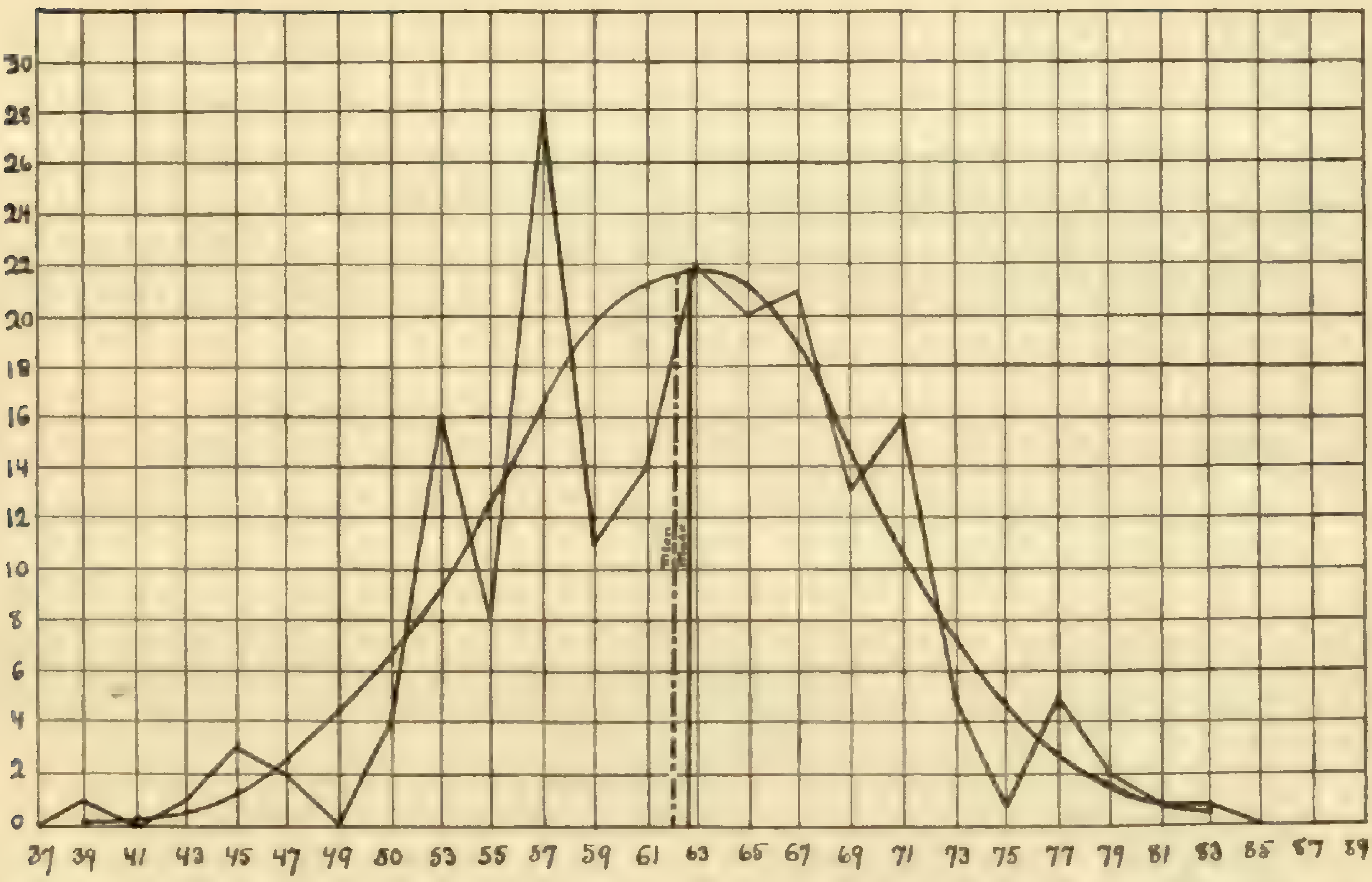

FIG. 3. FREQUENCY POLYGON AND FITTED CURVE FOR VARIATION IN LENGTH OF ZYGOSPORES PRODUCED BY LATERAL CONJUGATION (INBREEDING) IN *Spirogyra inflata* (Vauch.).

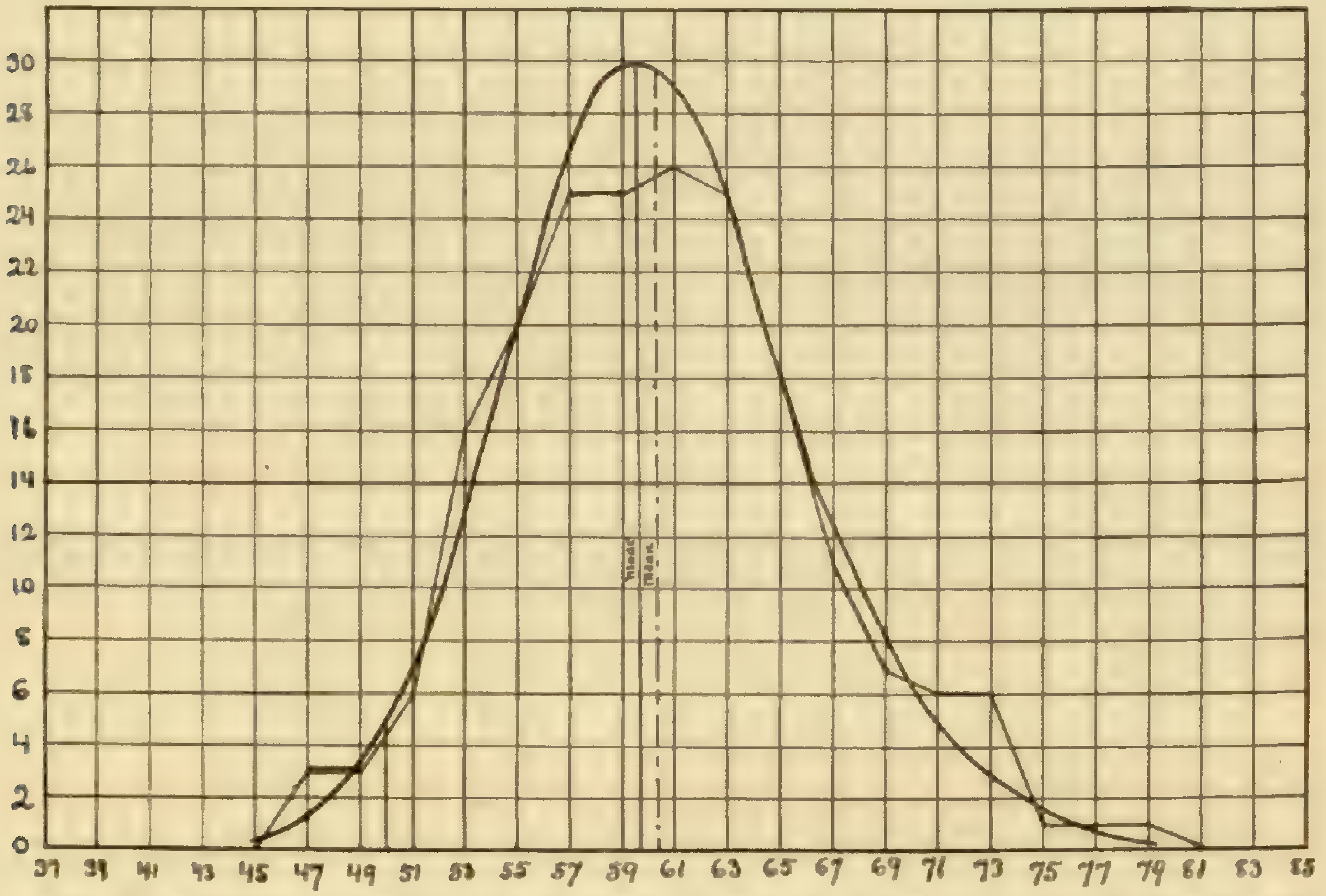

FIG. 4. FREQUENCY POLYGON AND FITTED CURVE FOR VARIATION IN LENGTH OF ZYGOSPORES PRODUCED BY SCALARIFORM CONJUGATION (CROSS BREEDING) IN *Spirogyra inflata* (Vauch.).

2. *Comparative Variability in the Diameter of the Zygospores*

The class range adopted in the frequency distribution for diameters (Table IV) of the two groups of zygo-

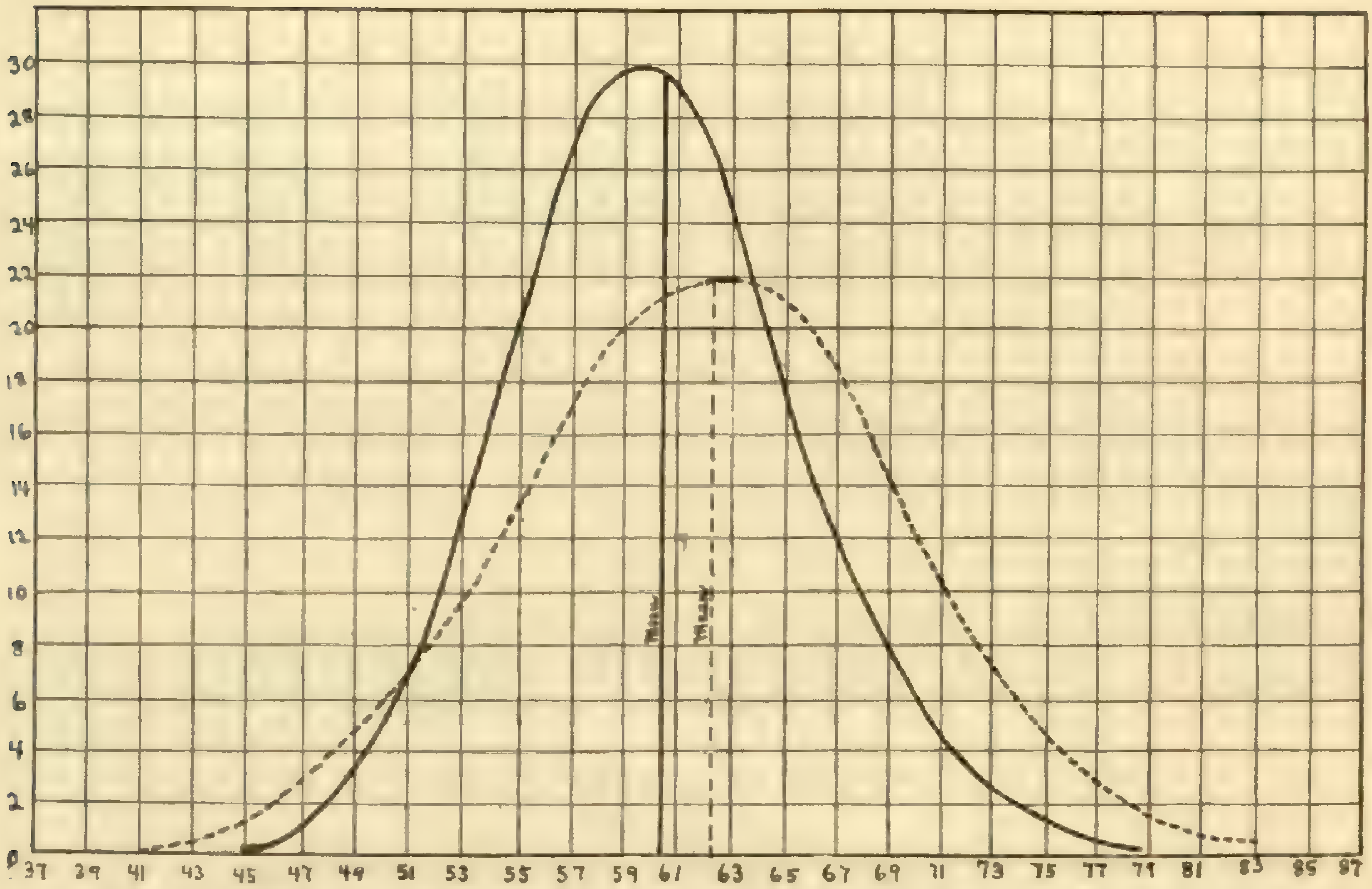

FIG. 5. COMPARISON OF FITTED CURVES FOR VARIATION IN LENGTH OF ZYGOSPORES PRODUCED BY LATERAL CONJUGATION (CLOSE BREEDING) AND BY SCALARIFORM CONJUGATION (CROSS BREEDING) IN *Spirogyra inflata* (Vauch.). Lateral conjugation = — — — — — — —. Scalariform conjugation = ————————.

spores was one micron, measurement being made at the maximum diameter. An inspection of the distribution shows at once the greater concentration of the variates

TABLE IV

DIAMETERS OF 400 ZYGOSPORES FROM *Spirogyra quadrata* (HAAS.) 200 PRODUCED BY LATERAL CONJUGATION AND 200 PRODUCED BY SCALARIFORM CONJUGATION, ARRANGED IN CLASSES ACCORDING TO FREQUENCIES. MAGNITUDES IN 1/10 μ.

DIAMETER OF ZYGOSPORES IN MICRONS.

Lateral Conjugation		Scalariform Conjugation	
Class	Frequency	Class	Frequency
23.0–23.9	0	23.0–23.9	1
24.0–24.9	3	24.0–24.9	0
25.0–25.9	4	25.0–25.9	3
26.0–26.9	12	26.0–26.9	9
27.0–27.9	22	27.0–27.9	13
28.0–28.9	44	28.0–28.9	38
29.0–29.9	40	29.0–29.9	46
30.0–30.9	25	30.0–30.9	46
31.0–31.9	16	31.0–31.9	30
32.0–32.9	15	32.0–32.9	9
33.0–33.9	10	33.0–33.9	4
34.0–34.9	9	34.0–34.9	1
Total......	200	Total......	200

TABLE V

GENERAL CONSTANTS FOR VARIATION IN DIAMETERS OF ZYGOSPORES BASED ON 200 FORMED BY LATERAL AND 200 FORMED BY SCALARIFORM CONJUGATION WITH A CLASS RANGE OF 1 MICRON.

Constant		Lateral Conjugation, Close Breeding		Scalariform Conjugation, Cross Breeding	
Name	Symbol	Value	Prob. Error	Value	Prob. Error
Number	N.	200	....	200	....
Mean	M.	29.66	±.1049	29.725	±.0801
Mode	Mo.	29.1577	....	29.9735	....
Standard deviation	σ.	2.1980	±.0688	1.6796	±.0583
Coefficient of variation	C.V.	7.5376	±.0339	5.7471	±.0338
Skewness	Sk.	.2285	±.0505	−.1480	±.0566

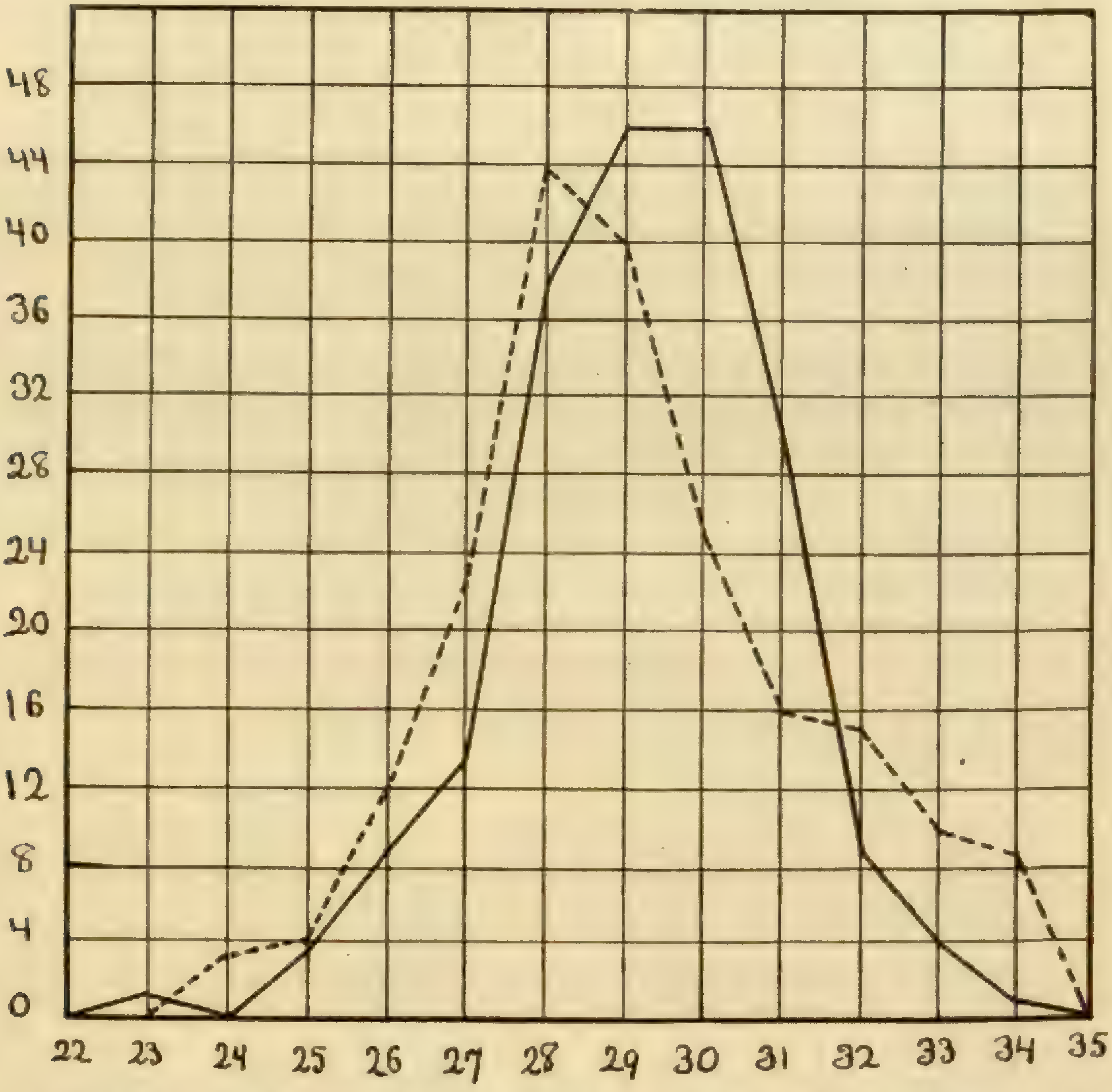

FIG. 6. DIAGRAM SHOWING THE FREQUENCY POLYGON FOR THE VARIATION IN DIAMETER OF ZYGOSPORES IN *Spirogyra inflata* (Vauch.) PRODUCED BY LATERAL CONJUGATION (INBREEDING) AND BY SCALARIFORM CONJUGATION (CROSS BREEDING). The unit of abscissa is one mikron. — — — — — — — = Lateral conjugation (close breeding). ——————— = Scalariform conjugation (cross breeding).

in scalariform conjugation, and thus their smaller variability. In considering the general constants of variability (Table V) based on the diameters it is to be noted that the means (M.) do not differ, as was found when considering length. The standard deviation (σ) and the coefficient of variation (C. V.) once more demonstrate the greater variability of the laterally formed zygospores. The values of the constants for skewness (Sk.) are not sufficient, however, when considered with the probable error, to be of importance. The frequency polygons (Fig. 6) illustrate conditions, although no curves have been fitted.

3. *Comparative Correlation of Length and Diameter*

In view of the results obtained in a consideration of the variability, it will be of some interest to ascertain whether

TABLE VI

CORRELATION BETWEEN LENGTH AND DIAMETER OF 200 ZYGOSPORES OF *Spirogyra inflata* (VAUCH.) PRODUCED BY LATERAL CONJUGATION

Diameter x / y Length	23.0–23.9	24.0–24.9	25.0–25.9	26.0–26.9	27.0–27.9	28.0–28.9	29.0–29.9	30.0–30.9	31.0–31.9	32.0–32.9	33.0–33.9	34.0–34.9	Totals
38.0–39.9	...	...	...	...	...	1	...	...	...	...	...	...	1
40.0–41.9	...	...	...	...	...	...	...	...	...	...	...	...	0
42.0–43.9	...	...	...	...	...	...	...	...	1	...	...	...	1
44.0–45.9	...	...	1	1	...	...	1	...	...	...	...	...	3
46.0–47.9	...	...	...	1	...	...	...	...	...	1	...	...	2
48.0–49.9	...	...	...	...	...	...	...	...	...	...	...	...	0
50.0–51.9	...	...	1	...	1	...	1	1	...	...	...	...	4
52.0–53.9	...	1	...	2	3	2	3	2	...	2	1	...	16
54.0–55.9	...	...	...	1	2	2	3	2	...	...	...	...	10
56.0–57.9	...	...	1	1	4	6	5	3	2	...	3	1	26
58.0–59.9	...	...	...	...	...	2	4	1	3	...	...	1	11
60.0–61.9	...	...	...	1	1	6	6	2	...	1	2	...	19
62.0–63.9	...	1	...	...	...	7	5	4	...	3	...	2	22
64.0–65.9	...	1	...	...	2	5	3	1	3	2	2	1	20
66.0–67.9	...	...	...	1	5	7	1	3	...	2	1	1	21
68.0–69.9	...	...	...	...	2	3	4	3	...	...	...	1	13
70.0–71.9	...	...	...	4	1	1	3	1	3	1	...	2	16
72.0–73.9	...	...	...	...	...	1	1	1	...	1	1	...	5
74.0–75.9	...	...	...	...	...	...	...	...	1	...	...	...	1
76.0–77.9	...	...	1	...	...	...	...	1	1	2	...	...	5
78.0–79.9	...	...	...	...	1	...	...	...	1	...	...	...	2
80.0–81.9	...	...	...	...	...	1	...	...	...	...	...	...	1
82.0–83.9	...	...	...	...	...	...	...	...	1	...	...	...	1
Totals........	...	3	4	12	22	44	40	25	16	15	10	9	200

the inbred zygospores produced by lateral conjugation will be more or less correlated than the cross bred zygospores produced by scalariform conjugation so far as length and diameter are concerned.

The value of perfect correlation as measured by the constant (r) is unity, while absence of correlation allows the value to become zero. Length is taken as the subject class (y) and diameter (x) as the relative class in the accompanying tables (VI and VII).

TABLE VII

Correlation between Length and Diameter of 200 Zygospores of *Spirogyra inflata* (Vauch.) produced by Scalariform Conjugation

Length y \ Diameter x	23.0–23.9	24.0–24.9	25.0–25.9	26.0–26.9	27.0–27.9	28.0–28.9	29.0–29.9	30.0–30.9	31.0–31.9	32.0–32.9	33.0–33.9	34.0–34.9	Totals
38.0–39.9	...	...	...	...	...	...	...	...	...	...	...	...	0
40.0–41.9	...	...	...	...	...	...	...	...	...	...	...	...	0
42.0–43.9	...	...	...	...	...	...	...	...	...	...	...	...	0
44.0–45.9	...	...	...	...	...	...	...	...	...	...	...	...	0
46.0–47.9	...	...	...	...	1	...	1	...	...	...	1	...	3
48.0–49.9	...	...	...	...	...	1	...	1	1	...	...	...	3
50.0–51.9	...	...	...	2	...	2	1	1	...	...	...	...	6
52.0–53.9	1	...	...	1	1	2	5	4	2	...	...	...	16
54.0–55.9	...	...	...	2	2	4	6	4	1	...	1	...	20
56.0–57.9	...	...	1	...	2	5	5	5	4	3	...	...	25
58.0–59.9	...	...	...	2	...	4	5	13	...	...	...	1	25
60.0–61.9	...	...	1	...	...	1	9	7	7	1	...	...	26
62.0–63.9	...	...	1	...	2	6	6	3	6	...	1	...	25
64.0–65.9	...	...	...	1	2	3	4	2	4	2	...	...	18
66.0–67.9	...	...	...	...	1	3	1	3	1	1	1	...	11
68.0–69.9	...	...	...	1	...	2	1	1	2	...	...	...	7
70.0–71.9	...	...	...	...	1	3	1	...	...	1	...	...	6
72.0–73.9	...	...	...	...	...	1	1	1	2	1	...	...	6
74.0–75.9	...	...	...	...	...	...	...	1	...	...	...	...	1
76.0–77.9	...	...	...	...	...	1	...	...	...	...	...	...	1
78.0–79.9	...	...	...	...	1	...	...	...	...	...	...	...	1
Totals........	1	0	3	9	13	38	46	46	30	9	4	1	200

While one might infer that the longer a zygospore the greater the diameter, such a condition is not apparent by mere inspection of the tables in either case. Consequently on solving the equations we are prepared to find that the coefficients have an extremely low value in each group.

Lateral Conjugation .... $r = .1894 \pm .0460$
Scalariform Conjugation .... $r = .0934 \pm .0473$

Although in lateral conjugation the value is more than four times the probable error, one is scarcely prepared to state that there is greater correlation between characters in close breeding than in cross breeding on the basis of the data noted above. When considered with the results presented in Table XII, the conclusion seems fully established, however.

4. *Comparative Size of Zygospores*

The term "size" as noted in the subsequent discussion is open to various interpretations dependent as to whether length, diameter or volume is being considered, a condition which to some extent complicates the interpretation of size characters among multicellular organisms which are in general dependent on the number rather than the dimensions of the component cells.

Those zygospores produced by lateral conjugation (close bred), so far as the present material is concerned, have an average length considerably exceeding those produced by scalariform conjugation (cross bred) while the diameter is approximately the same. This is illustrated in Table VIII.

TABLE VIII

COMPARATIVE LENGTH, DIAMETER AND VOLUME OF ZYGOSPORES PRODUCED BY LATERAL (CLOSE BRED) AND BY SCALARIFORM (CROSS BRED) CONJUGATION

Method Produced	Mean Length	Mean Diameter	Mean Volume
Lateral conjugation........	62.380 m. ±.178	29.660 m. ±.105	28,733 cub. m.
Scalariform conjugation.....	60.440 m. ±.135	29.725 m. ±.080	27,972 cub. m.
Differences favoring lateral conjugation.............	+1.940 m.	−.070 m.	+771 cub. m.

Consequently, here, the average zygospore produced by lateral conjugation has a greater volume than that produced by scalariform conjugation. Utilizing the formula

for computing the volume of a prolate spheroid ($V = 1/6\pi ld^2$) the difference is 771 cubic m. in favor of the former, although relatively this approximates only 3 per cent.

A question of some interest is at once suggested, namely, the possibilities for nourishment and development in cells of large and of small volume, inasmuch as one with a maximum volume has relatively less surface through which nourishment may be obtained. Thus growth may be retarded.

III. Discussion of Results

The close bred forms on the basis of the characters studied in the given population have been found more variable as to both length and diameter, more highly correlated, and larger taking into consideration length and volume. The value of the conclusions in their application to the solution of problems of evolution is dependent on the logical application of cause and result as well as the methods of the investigation.

That the two groups of zygospores are comparatively close bred and cross bred will scarcely be denied, particularly when it is remembered that in lateral conjugation nearly all adjacent pairs of cells in a filament had united in the process, each pair producing a zygospore, all pairs having originated from the same cell. With the material taken from a part of a mass a few centimeters square, a sample of a whole population has been utilized, and from what is known of the reproduction of *Spirogyra*, it may be assumed with reasonable certainty that the entire mass had its origin from zygospores produced in a few filaments the preceding year. With practically all zygospores measured in each filament, the criticism that isolated zygospores of mixed descent were studied, and that greater variability would be expected in those produced by lateral conjugation, loses its force. Furthermore it is believed that all investigations thus far

made, upon analysis support the direct conclusions which follow.

It may be objected that cells of mature filaments originating from the zygospores should have been studied. While this would have been of interest, the zygospores themselves are individuals in the cycle of development, and the differences as represented in the groups chosen can not be said to have less value than data from another part of the life cycle.

The possibility of the results being affected by the swelling of zygospores due to the 2 per cent. formalin used in preservation, became apparent when other duties prevented measurements within the anticipated time. The first series of 358 zygospores was measured between April 2 and May 16, while the remaining 42 were measured between August 17 and 21. The question seemed an important one, and in order to test the extent of such an error if present, the average diameter of the last lot was compared with that of the first lot, the values being 29.15 m. and 29.08 m., the difference of 0.07 m. being well within the limits of the probable error. The 42 zygospores measured August 17–21 happened to consist of an equal number of lateral and scalariform individuals, which would thus tend to eliminate an error should it have occurred. Consequently the use of the formalin does not appear to have affected the results.

Some evidence has been presented that new phylogenetic characters are more variable than older characters. Thus if lateral conjugation was a recent acquisition the greater variabitiy might have been expected. Pearl and Clawson ('07) found a higher variation in the great chela of the crayfish, *Camburus propinquus* Girard, than in the protopodites of the 2 and 3 legs, nevertheless they preferred to attribute the result to ontogenetic rather than to phylogenetic factors. MacDougall, Vail and Shull ('07) stated that

> the greater variability of phylogenetically new characters as compared with older ones . . . is confirmed . . . (p. 89).

The conclusion is open to objection inasmuch as they were comparing a hybrid with a single parental type and in general the greater variability would be expected. Consequently even admitting that lateral conjugation has been a more recent development than scalariform conjugation, it would not be demonstrated that an error had thus arisen.

1. *Comparative Variability*

Within the limits of the characters studied so far as the present material is concerned, it is evident that the zygospores produced by close breeding are more variable than those produced by cross breeding. While it is another proposition to extend the conclusion and insist that organisms produced asexually, by pure lines, or by close breeding, are more variable than those produced sexually or by cross breeding, it would seem that the facts strongly support such a conclusion and in connection with the evidence afforded by the investigations of Warren, Casteel and Phillips, Kellogg, and Wright, Lee and Pearson, it certainly may be denied that amphimixis or cross breeding as compared with other types actually produces variations, as has long been the prevalent belief.

The question here of particular interest, however, is that of the excess type of variability represented in *Spirogyra*. Inasmuch as the material was homogeneous in every way, it may be asserted that the greater variability exhibited by the close-bred forms is not fluctuability due to environment. It is also evident that, theoretically, cross breeding produces a greater number of combinations than inbreeding, nevertheless that the variability thus resulting is overwhelmed by that of another type in nature, is clear from the results noted in the preceding pages. An excellent demonstration of such condition is obtained by recalculating constants obtained by Hayes ('12) as shown in the accompanying table (Table IX) based on data obtained in connection with the breeding of *Nicotiana tabacum*.

TABLE IX

COMPARISON OF VARIABILITY OF *Nicotiana tabacum* IN COMBINED PARENTAL TYPES (NO. 3 AND 8) WITH VARIABILITY IN SEPARATE PARENTAL TYPES (NO. 1, 2, 6 AND 7), IN THE FIRST HYBRID GENERATIONS (NO. 4 AND 9) AND IN THE SECOND HYBRID GENERATIONS (NO. 5 AND 10). NO. 3 AND 8 CALCULATED FROM DATA BY HAYES IN TABLES NOTED. OTHER CONSTANTS AS GIVEN BY HAYES

No.	Table	Type	Character	S. D.	*E*	C. V.	*E*
1	XV	401	Number of leaves	0.96	±.037	5.00	±.189
2	XVI	403	" "	1.49	±.058	5.27	±.578
3	XV–XVI	401+403	" "	4.70	±.129	19.55	±.129
4	XVII	403×401	" "	1.30	±.056	5.51	±.215
5	XVIII	403×401−1−F_2	" "	2.24	±.103	9.40	±.551
6	XV	401	Height of plant	3.85	±.150	7.00	±.150
7	XVI	403	" "	4.55	±.177	5.98	±.177
8	XV–XVI	401+403	" "	11.31	±.311	17.35	±.312
9	XVII	403×401	" "	4.54	±.177	6.41	±.249
10	XVIII	403×401−1−F_2	" "	7.22	±.333	13.60	±.333

Here the constants of No. 3 and No. 8 have been obtained by combining the two parental types (401 and 403) both for the number of leaves and the height of the plant, and it may be noted that the coefficient of variation has dropped from 19.55 to 9.40 in the one case and from 17.35 to 13.60 in the other case. Thus variability as measured statistically has decreased. Those who have advocated an increased variability as the result of hybridization are correct when comparison is made of the F_2 generation with the F_1 generation or with a single parental generation. They are not correct, however, in making a general statement that cross breeding increases variability since the variability of the group composed of both parental types must be considered and upon so doing, it may normally be found that there has actually been a decrease in variability.

The possibility exists however that the variability will appear to have been increased when forms having the same phenotype but different genotypes are bred together. Such a condition may be illustrated by the two strains of white sweet peas crossed by Bateson which produced purple flowers in the first (F_1) generation, and purple,

pink, mixed and white flowers in the second (F_2) generation. New combinations had arisen, but only as an expression of that which already existed in the phenotypes, for there is no evidence of an increase in unit characters nor was there an actual increase in variability.

There are only three papers of a statistical nature in which it has seriously been asserted that cross-bred forms or conjugating forms produced greater variability than resulted in close-bred forms or non-conjugating forms.

The first is that of Castle, Carpenter, Clark, Mast and Barrows ('06) based on a series of observations as to the effect of cross breeding and close breeding on the variability and fertility of the small fruit fly *Drosophila ampelophila* Loew. In conclusion it was stated that "inbreeding did not affect the variability in the number of teeth of the sex comb of the male, nor the variability in size," the first opinion resulting from the value of the coefficient of variation in the number of tibial spines, the second from the standard deviation in the length of the tibia. In the former case the data certainly do not permit a clear conclusion one way or the other. In the second case, however, if the value of the coefficient of variation is computed for the length of the tibia—which, strange to say, was not done in the original investigation—and thus allowance made for the greater length of

TABLE X

ILLUSTRATING COMPARATIVE VARIABILITY OF CROSS BRED AND INBRED FORMS OF *Drosophila* AFTER COMPUTING THE VALUE OF THE COEFFICIENT OF VARIATION FOR THE LENGTH OF TIBIA FROM DATA BY CASTLE AND OTHERS

S.D. = Standard Deviation. C.V. = Coefficient of Variation.

Group		Spines of Sex Comb		Length of Tibia	
Generation	Number	S. D.	C. V.	S. D.	C. V.
Cross bred (*X*-1)	100	1.749 ± .083	16.23	1.461 ± .070	3.531 ± .168
Inbred (*M*-31)	100	1.568 ± .075	15.51	1.723 ± .082	4.452 ± .212
Inbred (*N*-30)	100	1.684 ± .080	17.38	2.842 ± .136	8.167 ± .389
Inbred (*A*-61)	100	1.857 ± .089	17.60	2.041 ± .097	5.245 ± .250

tibia in the cross-bred forms (Table X) the average variability of the three inbred groups is 68 per cent. greater than that of the cross-bred group. Consequently, the results decidedly support the facts in the present paper.

The remaining papers are those of Jennings ('11 and '13) in a study of *Paramecium*. In the first paper the breeding experiments are summarized as follows:

> The progeny of conjugants are more variable, in size and in certain other respects, than the progeny of the equivalent non-conjugants. Thus conjugation increases variation.

It seems difficult to account for this conclusion if one subjects the data to a critical review. So far as a "pure race" is concerned the non-conjugants and their progeny were decidedly *more variable* than the conjugants and their progeny (Table 28, p. 94), although the small number utilized March 31 for the statistical work (42 and 34) is not sufficient to justify a conclusion in either direction. Even in a "wild culture" (Table 32, p. 99) the evidence is too conflicting to justify a definite expression of opinion. Of the seven comparisons here made among the progeny, five showed an excess variability for the conjugants, but in only one case did the difference exceed three times the probable error, while two cases showed an excess variability for the non-conjugants, the difference in one case exceeding twice the probable error. Data from numbers so small (22–95) can scarcely be considered reliable. The comparison of the variability of "all pairs" and "all unpairs" on June 22 and June 23 denotes an excess variability for those completing conjugation at the beginning of the experiment.

In the second paper Jennings concluded (p. 363) that conjugation increased the variation in the rate of reproduction. The variation was increased, but the explanation of such increase seems comparatively simple when it is noted that among the conjugants there were many with a low rate of fission with death occurring. As com-

pared with the more normal rate of fission among non-conjugants, this could result in nothing but an increased variability, having, however, no bearing on the question at issue.

At the present time, therefore, it would seem that the preponderance of evidence demonstrates that variability is decreased in cross breeding.

2. *Comparative Size*

The zygospores produced by close breeding have a mean length of 62.38 μ. $\pm$ .18 μ. with a mean diameter of 29.66 μ. $\pm$ 10 μ. and those produced by cross breeding have a mean length of 60.44 μ. $\pm$ .13 μ. with a mean diameter of 29.725 μ. $\pm$ .08 μ. Thus so far as length is concerned the close bred zygospores are relatively 3.2 per cent. larger and although slightly smaller in diameter, when volume is considered by utilizing the formula ($V = \frac{1}{6}\pi l d^2$) the close bred forms are also 2.8 per cent. larger. Since these results are not in accord with the general belief that cross fertilization increases size and vigor, terms which have a diverse usage, however, it will be well to consider other evidence bearing on the problem with a view of attempting an explanation which may meet the conditions imposed.

Pearl ('07) in studying the conjugation of *Paramecium* with particular reference to assortative mating, notes that "the conjugant individuals when compared with the non-conjugant, are shorter and narrower" and stated in accordance with Calkins ('02) that the reduction in size was quite probably dependent on functional changes connected with reproduction. In *Spirogyra,* however, both the close-bred and the cross-bred zygospores go through similar reproductive processes in consequence of which one may question the theory that the method of conjugation is the decisive factor in bringing about the result even in *Paramecium.*

Jennings ('11) in comparing the size of conjugant and non-conjugant *Paramecium* stated that

The progeny of conjugants . . . were a little larger than the progeny of non-conjugants and the difference appears to be significant.

This conclusion was based on measurements of length and diameter, the volume not being computed. When this is done as shown in the accompanying table (Table XI) by

TABLE XI

COMPARISON IN SIZE OF CONJUGANT AND NON-CONJUGANT FORMS OF *Paramecium aurelia* AND THEIR PROGENY BASED ON VOLUME ($V = 1/6\pi ld^2)r$ FROM LENGTH AND DIAMETER MEASUREMENTS BY JENNINGS, 1911

Experiment			Non-conjugants and Progeny				Conjugants and Progeny				Non-conj. Exceed the Conjugants in Volume
Culture		Date, 1908	No.	Length Microns	Diam. Microns	Volume Cub. Microns	No.	Length Microns	Diam. Microns	Volume Cub. Microns	
	Nf?	Mar. 31	34	144 59	34	87,518	48	136.95	35.52	90,471	− 2,953
a	Table	Apr. 10	65	137.97	44	139,859	61	148.20	42.30	138,844	+ 1,015
	28	Apr. 20	103	156.48	43.82	157,327	108	160.85	42.04	148,849	+ 8,478
	C_2	Sept. 16	110	132.18	?	?	138	121.91	?	?	+ ?
b	Table	Sept. 18	70	116.17	31.20	59,211	15	128	31.20	65,241	− 6,030
	29	Sept. 26	52	122.15	34.81	77,500	11	112.36	29.50	51,198	+ 26,302
	g	Sept. 27	118	135.35	?	?	174	118.28	?	?	+ ?
c	Table	Sept. 29	10	156.40	49.60	201,465	6	135.33	36	91,833	+109,633
	30	....	...	......	......	......	...	......	......	......	+
	k	Sept. 12	100	140.20	?	?	336	129.58	?	?	?
d	Table	Oct. 28	10	136	37.60	100,673	39	131.38	35.49	86,644	+ 14,029
	31	Oct. 30	28	123.71	34.14	75,497	25	128.16	36.32	88,520	− 13,023

utilizing the formula $V = 1/6\pi ld^2$, thus allowing for slight decreases in diameters, the facts present a different interpretation.

Three (*a, b, c*) of the four experiments dealing with a "pure race" of *P. aurelia* indicate that the progeny of the non-conjugants become larger, even when as a group they are smaller (*a, b*?) at the beginning of the experiment. While the fourth (*d*) indicates a reverse condition so far as the measurements of October 30 are concerned, the measurements of the sixth and seventh generations immediately preceding, demonstrate that the non-conjugants were larger. The result on October 30, where the non-conjugants became smaller, may have depended

on the elimination suggested by "all existing progeny." The extraordinary diminution in length (140 μ. to 123.71 μ.) suggests some disturbing factor of metabolism.

The results of the experiment with a "wild culture" where progeny of "unpaired" and "paired" forms of *P. caudatum* (?) were considered, again suggested to Jennings the greater size of the progeny of the paired individuals (conjugants), a condition which was particularly evident in the first generation. But it must be noted that the disturbance of the function of conjugation in "unpairing" may have produced the result. The progeny of the "unpairs" were relatively becoming larger from the first to the seventh generation. These facts taken together with the absence of measurements of mean diameters by which to calculate the mean volumes, suggest that such a conclusion based on that part of the work could not be accepted, and that the data strongly support the proposition directly contrary to Jennings that the progeny of conjugants tend to become smaller than the progeny of non-conjugants although the latter may be larger directly after conjugation as a result of slower fission. Thus the evidence from various sources, although incomplete, suggests that cross-bred unicellular organisms are smaller than close-bred forms.

Among multicellular organisms however it has long been recognized that hybrids usually grew to a larger size than either parental form, as has been observed by Kohlreuter ('63), Knight ('99), Gärtner ('49), as well as Darwin, Mendel and others, although the cause of the increased growth has been purely conjectural. It is quite evident that the result is due to either the increased number of cells, a suggestion made by East, to the increased size of the cells, or to the combination of both conditions.

The question immediately arises as to the cause of the increased size and vigor among cross-bred multicellular organisms when the evidence indicates that cross-bred unicellular organisms are smaller instead of larger.

Some investigations in progress[3] suggest an answer meeting the conditions, although more than a provisional opinion may as yet not be ventured. This is to the effect that the cells of cross-bred multicellular organisms are actually smaller than the cells of pure line or inbred organisms, and that the more rapid division is a function of the greater ratio surface has to volume in a small cell with the better opportunity this afforded for an increased metabolism.

The increase of size in plant and animal forms to the physiological limit has great importance for the future of agriculture and stock breeding, but many subsidiary problems must be solved before practical results are attained in this direction. The relative rate of growth, number and size of the constituent cells of pure line and of hybrid individuals is one of the problems.

3. Comparative Correlation Resulting from Close Breeding and Cross Breeding

The close-bred zygospores are more correlated as to length and diameter than the cross-bred zygospores, but since the difference only slightly exceeds twice the probable error, the value of the result here is questionable. Considering other investigations (Table XII), it may be noted that the group containing close-bred, asexual or non-conjugating organisms, is more highly correlated in respect to characters than the group consisting of cross-bred, sexual, or conjugating organisms, although two exceptions, No. 12 and No. 14, are presented. An interesting fact, although possibly only a coincidence, is that cross bred zygospores of *Spirogyra* and of conjugating *Paramecium* have approximately only one half the correlation exhibited by close bred zygospores of *Spirogyra* and by non-conjugating *Paramecium*.

The explanation of the conclusion here reached, that the value of a character "x" in cross-bred forms does not have the same tendency to change that the value of a

[3] Walton ('14).

TABLE XII

COMPARATIVE CORRELATION OF CHARACTERS IN CROSS-BRED AND CLOSE-BRED ORGANISMS INCLUDING CONJUGANT AND NON-CONJUGANT *Paramecium*, AND PARTHENOGENETIC AND SEXUALLY PRODUCED WASPS

	Organism	Authority	Characters Compared	Type of Development	Value of "r"
1	*Paramecium*	Pearl, '07	Length and	Non-conjugants, Ser. *A*	.589±.03
2	"	" "	diameter.	Conjugants.	.278±.04
3	*Drosophila*	Barrows, '06	Number of	Close bred, Ser. *A*–61.	.469±.05
4	"	" "	spines and	" " Ser. *M*–31.	.448±.05
5	"	" "	length of	" " Ser. *N*–30.	.708±.03
6	"	" "	tibia.	Cross bred, Ser. *X*–1.	.141±.07
7	*Nicotiana*	Hayes, '12	Number of	Close bred, No. 401.	.368±.05
8	"	" "	leaves and	" " No. 403.	.631±.03
9	"	" "	height.	Cross bred, 403×401.	.406±.05
10	"	" "	Length and	Close bred, No. 401.	.684±.03
11	"	" "	breadth of	" " No. 403.	.497±.04
12	"	" "	leaf.	Cross bred, 403×401.	.818±.02
13	*Vespa vulgaris*	Wright, Lee,	Length and	Drones.	.772±.02
14	"	and	breadth of	Workers.	.912±.01
15	"	Pearson, '07	r. wing.	Queens.	.558±.04
16	*Spirogyra*	Walton, '15	Length and	Close bred (Lat. *C.*)	.189±.05
17	"	" "	diameter.	Cross bred (Scal. *C.*)	.093±.05

related character "*y*" has in close-bred forms, apparently rests on a Mendelian basis. Its importance in evolution, beyond the idea that more pronounced temporary combinations are thus allowed in the trial and error plan of nature, is conjectural.

4. *Amphimixis and Death*

With the assumption that the results obtained in the preceding investigation, together with the data presented by other writers, when correctly analyzed, strongly supports the view that asexually produced organisms tend to be more variable than those produced by the union of two gametes, there is furnished evidence for the interpretation of the origin of sex—amphimixis and also for the origin of death that would seem to rest upon a much more secure basis than the purely speculative theories of Weismann, Nägeli, Hatscheck, Metschnikoff, Minot, etc., which have previously been advanced.

The chief advantage gained in the reduction of variability, while somewhat conjectural, would appear to be

that of holding organisms within limited bounds, or in other words, *asexually produced organisms in general tend by their variability to exceed the limits of their environment and thus perish, while organisms produced by the mingling of two diverse lines of germ plasm with their lessened variability meet the conditions of the comparatively slowly changing environment and their race persists.* This idea was proposed entirely upon speculative grounds by Hatscheck ('87) who suggested that variation would run riot if not controlled by the union of germ cells, and it would now appear that the facts support such a proposition. While it has been suggested that the chief function of amphimixis was that of rejuvenation, a consideration of the discussion on "Comparative Size" as well as the recent experimental results obtained in the production of *Paramecium* do not support such an opinion to the exclusion of the hypothesis here put forward. East and Hayes ('12) have advanced the theory that recombinations in accordance with Mendelian principles were the chief purpose of amphimixis. While new combinations are thus brought about, apparently there exists a real difficulty in understanding how transitory heterozygotic forms could become of selective value in originating and maintaining such a process.

The acceptance of the conclusion that asexually produced organisms are more variable than those produced by amphimixis, and that thus some of the units are more readily subject to the eliminating influences of the environment, affords a comparatively simple explanation of the origin of death in multicellular forms which are built up of such units—the cell. Consequently the inference is that[4] *death occurs as the result of the continually forming body cells becoming so variable through the absence of control by amphimixis, that eventually some one group fails to meet the limits imposed by the environment, and these together with the remainder of the colony*

[4] Walton, *Science*, p. 216, 1909.

—*the individual—perish.* The experiments of Woodruff ('11, etc.) who in extending the work of Maupas and of Calkins was able to rear several thousand generations of *Paramecium* without conjugation, as well as the investigations of Harrison subsequently elaborated by Carrel, where human and other animal tissues maintained cell division for a prolonged time in an artificial medium, are here of much interest. In each case the result is brought about by the favorable artificial environment, and it is made more clear that death itself is wholly or in part due to the unfavorable conditions surrounding an organism.

IV. A Working Hypothesis of Evolution

Investigations during the last fifteen years, instead of establishing evolution as the simple process of natural selection conjectured by Darwin and others, have made it evident that the results are due to many factors of much complexity. While the diversity of organisms depends on variation—their inheritance and non-inheritance—it is becoming more and more apparent that the term is too comprehensive and covers variations arising in organisms from causes quite different from one another.

The results reached in the preceding pages indicate the need of extending the older terminology as used by Plate, '13, and others where variations are separated into "somations" or fluctuations induced by the environment and not inherited, and "mutations" or blastovariations arising in the germ plasm and inherited, if a clearer understanding is to be obtained of evolution and its application. Therefore the following scheme is proposed.[5]

[5] Several interesting groupings of variations have been suggested by Spillman, Baur and others, none of which, however, appear to meet present conditions.

VARIATIONS

*A*1. Apparently originating in accordance with definite laws ....*A*. **Normations.**

*B*1. Induced by general environmental stimuli, (food supply, use and disuse, etc.), but not inherited so far as evidence shows............ 1. *Fluctuations.*

*B*2. Not induced by environmental stimuli; inherited.

*C*1. Arising through the transference of factors by the combination of two ancestral lines in accordance with Mendelian principles, but exhibiting "per se" no definite progress...... 2. *Amphimutations* ("mutations" in part).

*C*2. Arising through causes at present unknown, but which, from the progressive results obtained, may be assumed to originate in accordance with definite laws .... 3. *Cumulations.*[6]

*A*2. Apparently not originating in accordance with definite laws ....*B*. **Abnormations.**

*B*1. Induced during early developmental stages of the embryo from intracellular (?) stimuli, and inherited.

*C*1. Arising through the abnormal segregation of the hereditary material (chromosomes?) . . 4. *Malsegregations* ("mutations" in part).

*C*2. Arising by the loss of hereditary qualities.

*D*1. Resulting from the functional loss of a factor controlling a character .... 5. *Defactorations* ("mutations" in part).

*D*2. Resulting from the partial functional loss of a factor controlling a character.... 6. *Fractionations* ("mutations" in part).

*B*2. Induced during the early developmental stages of the embryo from extracellular (?) stimuli and not inherited .... 7. *Malformations.*[7]

While any scheme presented must change as new facts are obtained, a terminology is of value in proportion as it gives a basis for future progress. The objection that it is not possible to point out a specific cumulation by no means indicates the absence of such progressive variations taking long intervals of time, by the haphazard

[6] Cumulations—from cumulo, to increase—including the names of the following groups, with the exception of fractionations proposed by Bateson, are based on the apparent origin of the variations.

[7] Many so-called malformations originate as defactorations, etc.

method of nature, in which to bring about a change evident to mankind. That the weight of evidence, so far as investigations have gone, is against evolution by means of the other variations noted, makes the explanation the more plausible. While it is true that Bateson ('14) has urged the consideration of the proposition that organic changes occur through the loss of inhibiting factors—defactorations—such a double negative theory assumes a decreasing complexity instead of an increasing complexity of protoplasm, as already pointed out by Castle, ('15) and seems impossible to maintain.

On the interpretation here presented, the diversity of organic forms is more complex than earlier imagined, and the problem of positive racial improvement is still far from solution. Loss as well as segregation factors may add new forms which really contain nothing new. To build up and not to break down is the desideratum, and the data obtained would seem to suggest that pure line breeding with the employment of statistical methods to show any progress would be the path leading most directly to the goal.

VI. Conclusions

1. *Direct Conclusions*

The following conclusions drawn from the investigation are primarily statements of fact.

1. Zygospores of *Spirogyra inflata* (Vauch.) produced by lateral conjugation or close breeding (quasi-parthenogenesis) are relatively 26 per cent. more variable in length and 31 per cent. more variable in diameter as measured by the coefficient of variation, than those produced by scalariform conjugation or cross breeding (sexual reproduction).

2. The size (volume) is greater in the average (mean) zygospore close bred by lateral conjugation, where the mean length is 62.38 μ. $\pm$ .178, than in the average zygo-

spore cross bred by scalariform conjugation, where the mean length is 60.44 μ. $\pm$ .135, The diameter is approximately the same in both types.

3. In zygospores produced by lateral conjugation there exists a positive correlation between length and diameter of .1894 = .0460, while in scalariform conjugation the value is .0934 = .0473. This is in general agreement with results obtained by others although here the difference is not significant when the probable error is considered.

4. In the material studied approximately 45 per cent. of the zygospores were formed by lateral conjugation, the remaining 55 per cent. by scalariform conjugation.

5. The material studied was strictly homogeneous, and evidently arose from the same parental stock, both types of filaments being intermingled with no structural differences except those of conjugation. Consequently the differences in variability are not the result of fluctuability.

2. *Indirect Conclusions*

The conclusions here presented are generalizations based on the present investigation as well as the work of others, and represent propositions concerning which differences of opinion may exist.

1. Amphimixis, cross-breeding, etc., decreases and does not augment variability (cumulability) although amphimutability may temporarily be increased.

2. Close bred forms are more highly correlated in respect to related characters than cross-bred forms.

3. Variations, so far as their origin is concerned, may be separated into (*A*) Normations consisting of (1) fluctuations, (2) amphimutations, and (3) cumulations, and into (*B*) Abnormations consisting of (1) malsegregations, (2) defactorations, (3) fractionations, and (4) malformations.

4. Cumulations may best be investigated among organisms produced asexually, by pure lines, or by close breeding than by cross breeding, etc.

5. Sexual reproduction and cross fertilization have been advantageous in the evolution of organisms by limiting cumulability and thus confining the progress of the group to a path bounded by the more permanent environment.

6. Death occurs as a result of the continually forming body cells becoming so variable through the absence of control by amphimixis, that eventually some one group fails to meet the limits imposed by the environment, and these together with the remainder of the colony—the individual—perish.

3. Hypotheses

The following opinions in the nature of hypotheses based to a large extent on the preceding work may be confirmed or invalidated by future investigations.

1. Variability (cumulability) will be greater in a small and isolated population than in a large and less isolated population.

2. Progressive evolution has resulted from factors arising through cumulations without reference to amphimutations (Mendelian combinations).

3. Characters once established by cumulations produce by fluctuations, amphimutations, etc., the diversity of organic life. Such secondary variations are only indirectly the products of evolution.

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GENETIC STUDIES OF SEVERAL GEOGRAPHIC RACES OF CALIFORNIA DEER-MICE[1]

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SOME of those present may recall a resolution which was adopted at a meeting of the Biological Society of the Pacific, held in Berkeley, in April, 1913, endorsing a project for the study of certain problems, related both to genetics and to geographical distribution. During the same year, the Scripps Institution for Biological Research found it possible to undertake the execution of this project, and the author of the present paper was chosen to carry it out. It is my object to-day to offer a preliminary report upon the results of these studies.

To those who have been so fortunate as to work in fields which yield quicker returns than does that of experimental breeding, it may seem that something more than a "preliminary report" might reasonably be expected after the lapse of a year and a half. If any justification is needed for such seeming slothfulness, I need only remark that my studies have already necessitated the trapping of about 600 living mice, of my chosen species, in four widely distant parts of the state, together with the rearing of several hundred others which were born in captivity; and that I have made measurements of some 500 of these animals, including skeletal measurements of over 400. Care of this rather large family of pets, statistical treatment of the measurements, continuous meteorological observations at several points, and the preparation of a certain number of skins and color photographs, are also to be included in the technique of this

[1] Read before a joint meeting of the American Association for the Advancement of Science (Section F), the American Society of Naturalists, the American Society of Zoologists, the American Genetic Association, and the Eugenic Research Association, at Stanford University, August 4, 1915.

project. Without the generous opportunities afforded me by the Scripps Institution, the work could never have been undertaken. And of an importance only second in order I must mention the assistance rendered me throughout these studies by the Museum of Vertebrate Zoology at Berkeley.

The resolution to which I have referred above formulated four questions which were regarded as especially worthy of consideration in the investigations contemplated. These were:

1. To what extent do influences such as external conditions, the exercise of organs or faculties, etc., which produce modifications of structure or function in the parent, result in bringing about parallel changes in the offspring?

2. If such changes are, in reality, found to reappear in the offspring, do they constitute true examples of heredity?

3. Are the subspecies or geographical races of the systematic zoologists fixed, in the sense of being hereditary, or do the differences by which they are distinguished depend upon conditions which must act anew during the lifetime of each individual?

4. If these subspecific characteristics are actually found to "breed true," do they owe their existence at the outset to "mutations" or to the cumulative effect of environmental influences, or to the mere fact of isolation, acting in some way independently of those influences?

To a large section of experimental breeders in this country, to whom "genetics" is synonymous with Mendelism, such a formulation of problems as this doubtless seems hopelessly archaic. "What is the use of raising all these dead issues," they will ask, "as if Weismann and De Vries and Johannsen had never lived?" And as for the question of subspecies, I suspect that some of our critics would grant them no existence whatever, outside the overwrought imagination of certain taxonomists.

Those, however, who have read dispassionately such able compilations of evidence as are offered us, for

example, by Plate[2] and Semon[3] are not likely to fall into the shallow dogmatism which dismisses the whole "acquired characters" question as once for all settled. And those who have taken the trouble to carefully examine a few trays of specimens, representing the subspecies of some widely ranging bird or mammal, will not so readily resort to a subjective interpretation of the phenomenon of geographic variation.

I shall give chief attention to-day to the case of a single species of white-footed mouse, or deer-mouse of the genus *Peromyscus*. According to Osgood,[4] the chief monographer of this genus, the species *maniculatus* comprises about 40 distinguishable geographic races, many of which are so unlike that they would be given full specific rank but for the fact that they intergrade insensibly with one another.

My own special studies have had to do chiefly with those subspecies of *Peromyscus maniculatus* which fall within the limits of the state of California. The first investigations have naturally been directed toward a careful examination of mice representing each of these local races, together with a determination, so far as possible, of the meteorological conditions to which they are subjected in nature. A search for correlations of any sort between structural and environmental differences was, of course, early undertaken.

Mice were collected at four points within the state: Eureka, Berkeley, La Jolla, and in the Mojave Desert near Victorville. At Eureka, Berkeley and Victorville, self-recording instruments (thermographs and hygrographs) have been left in charge of assistants for nine to fifteen months, and recording instruments will be installed at La Jolla this summer. It is planned to continue these records for at least two years. The instru-

2 "Selektionsprinzip," vierte Auflage, Engelmann, 1913.

3 "Das Problem der Vererbung 'erworbener Eigenschaften,' " Engelmann, 1912.

4 "Revision of the Mice of the American Genus *Peromyscus*," U. S. Department of Agriculture, Biological Survey, 1909.

ments are placed in positions more nearly representing the natural environmental conditions of the animals than is customary for regular Weather Bureau stations (*e. g.*, in a redwood forest on the outskirts of Eureka).

The Eureka mice are assigned to the subspecies "*rubidus*," those from the desert to "*sonoriensis*," while those

Distribution of the Subspecies of *Peromyscus maniculatus* in California and Nevada, Based upon the Distribution Map of Osgood (1909). The heaviest shading denotes the range of *P. m. rubidus*, the intermediate shading that of *gambeli*, the lightest that of *sonoriensis*. Areas of intergradation between two races are indicated by dotted lines.

from Berkeley and La Jolla are assigned by Osgood to the same subspecies "*gambeli,*" although, as I shall point out, there are certain slight differences of type between the two.

Now, as to characters, I have made 14 measurements of each completely measured mouse. Certain color characters, not capable of quantitative expression, have also been taken into consideration. I shall first consider the measurable parts. I must introduce this discussion by stating that my comparisons are entirely between animals of the same body length. When I say that *P. m. rubidus* has a longer tail than *sonoriensis,* I mean that this is true for mice of equal size. Owing to the impracticability of giving you a mathematical justification of all the steps which I have taken, I will ask you to credit me with a knowledge of the more elementary statistical methods.[5] I must also explain that I have thus far failed to kill and measure many animals from which I shall before long have full data. At present these are being retained for breeding purposes. Hence my series of measurements, in certain cases, is very small.

To present these subspecific characters briefly, I may say that, in respect to tail and foot length, *rubidus* stands in a class by itself. It does not require the trained eye of a systematist to detect the fact that this northern race has conspicuously longer tail and feet. In the case of the tail, this difference is due almost wholly to a difference in the length of the individual vertebræ, not to an increase in the number of these.[6] The other three races (*sonoriensis* and the two lots of *gambeli*) show no statistically certain differences in either of these characters. *P. m. rubidus* likewise has a significantly greater skull length and probably also a greater cranial capacity.[7]

[5] The detailed data must be deferred until a somewhat later stage of the work. Some of these were presented to the meeting in the form of graphs.

[6] The same was found to be true of the artificially induced modifications in tail length, described by me elsewhere for white mice.

[7] Determined by suitably cleaning and then desiccating the skulls, and weighing the volume of mercury which just filled the cranial cavity.

The La Jolla race of *gambeli* seems to fall second in the list in this regard.

The only significant difference in ear length is that between the two races of *gambeli,* the La Jolla stock having noticeably longer ears than the Berkeley stock, while *rubidus* and *sonoriensis* appear to be intermediate in this respect.

As regards color differences, these relate chiefly (1) to the depth of shade, and (2) to the extensity of the pigmented areas. A careful comparison of large numbers of the Berkeley race (*gambeli*) and the desert race (*sonoriensis*) revealed at least ten recognizable differences of this class, though in many cases these were merely different expressions of the same fundamental difference. None of the distinctions between these two races are absolute ones, holding between any two individuals of the contrasted races. Rather they are distinctions "on the whole," expressed by differences of mode or mean. Taken collectively, however, it is likely that these characters form an ensemble sufficiently distinct to reveal the identity of practically every specimen.

The most widely separate of the races, in respect to color, are *rubidus* and *sonoriensis,* the former race being very much darker than the latter. The two lots of *gambeli* occupy intermediate positions between the others.

METEOROLOGICAL DATA AT FOUR CALIFORNIA STATIONS.

	Temperature (°F.)		Humidity		Rainfall
	Annual Mean	Annual Range	Annual Mean	Daily range	Annual total
Eureka[8]	51.6	8.9	86	Very small	45
Berkeley	56.1[9]	14.4[9]	83[8]	Small	26[9]
La Jolla (San Diego)[8]	60.6	14.7	75	Small	10(−)
Mojave Desert	(Mojave)[8] 63.6	(Mojave)[8] 40.4	(Victorville)[10] 45±	High	(Victorville)[11] 6

[8] U. S. Weather Bureau.

[9] University Observatory.

[10] Computed from observations during present experiments (only one half year at this station).

[11] Rain-gauge records of Mr. Reginald Frost.

Now do we find any instances of correlation between these differences of structure or color and differences in environmental conditions? The most conspicuous of the structural differences relate to the greater length of the tail and foot of the Eureka race (*rubidus*) as compared with any of the other three races here considered. It is of considerable interest to note that there is here an increase in the length of these appendages as we pass to the northward, a circumstance which is still further emphasized by the condition of certain Alaskan subspecies. In fact, so far as these coastal subspecies of *Peromyscus* are concerned, there seems to be, within certain limits, a reversal of Allen's principle of the shortening of "peripheral parts" as we pass from south to north. The facts here revealed are likewise out of harmony with my own experimental results from white mice, which showed conclusively that low temperature and high humidity led to a decrease, rather than an increase in the length of the tail and foot.[12] A little later I shall point out the pronounced effect of certain other artificial influences upon the length of these appendages in *Peromyscus*, though I must admit that these later experimental results furnish no more satisfactory clue to the origin of these differences in nature.

On the whole, then, these preliminary researches do not offer much ground for believing that the differences found in the tails and feet of these wild races of *Peromyscus* result directly from any differences in environmental stimuli, or for expecting that they will respond appreciably to artificial climatic changes.

Passing to color differences, we seem to have here a good illustration of that correlation between atmospheric humidity and depth of pigmentation which has long been recognized to hold for mammals, birds and some other animals. If we arrange our four environments in ascending order with respect to their atmospheric humidity

[12] Cf. *Journal of Experimental Zoology*, April, 1915, and earlier papers therein cited.

(the same order holds with respect to their rainfall), we have the series: (1) Victorville, (2) La Jolla, (3) Berkeley and (4) Eureka. Correspondingly, the desert mouse (*sonoriensis*) is the palest of the lot, while the La Jolla mouse, the Berkeley mouse and the Eureka mouse follow in the order of increasing pigmentation. This relation, when viewed in connection with a wide range of known facts, and with certain experimental data to be noted later, can hardly be regarded as accidental. Any exact quantitative determination of the density of pigmentation would of course be difficult, and it has not yet been attempted. But the width of the dorsal median stripe of the tail is found to serve in some measure as an index of the extension of the darkly pigmented areas. It is interesting to note, in order of increasing width: *sonoriensis* (28 per cent.), *gambeli* (32 per cent.) and *rubidus* (43 per cent.). (The *gambeli* considered are from La Jolla.)

Let us grant then, provisionally, some sort of causal relationship between atmospheric humidity and the quantity of pigment in the hair or feathers. Now, aside from our ignorance of the physics and chemistry of the processes here involved, there is still a most important biological question left unsolved: Are these differences in pigmentation between the various geographical races germinal in their origin or are they purely somatic and individually acquired?

It was but a few years ago that Mr. J. A. Allen[13] was shocked by the very moderate suggestion of President Jordan's[14] that perhaps some of our subspecific differences were "ontogenetic," and not racially fixed. Mr. Allen was shocked, though unable to offer any really substantial evidence in reply. In this uncertainty over so elementary a matter of fact, everybody suggested decisive experiments for some one else to perform, but somehow no one seemed disposed to perform them. At least the

[13] *Science*, January 26, 1906.

[14] *Science*, December 29, 1905.

question of the fixity of the subspecies of mammals and birds has, to my knowedge, never before been put to experimental test.[15]

Now, in view of the subject matter of the present paper, it would ill become me to underrate the value of such tests. But I think that I have had enough to do with the experimental method in zoology to make me realize its rigid limitations. It is seldom indeed that we are able to perform a really crucial experiment and to obtain unequivocal results. Moreover, the mills of the gods grind slowly, while the single human life is short.

I am therefore disposed to attach considerable importance to what have been called "Nature's experiments." Certain of these have been cited by Grinnell and Swarth[16] in their bearing on the subspecies question. For example, two well-marked local races or subspecies of song-sparrow occur in southern California, on opposite sides of the high mountain range which divides the coastal plain from the desert interior. These races are separated nearly everywhere by the mountain barrier, but at certain points passes through the latter occur, permitting of migration from one side to the other.

Now these authors find localities in which the coast form of song-sparrow has penetrated, for considerable distances, into the desert and has become established there. Nevertheless, these invaders, which have perhaps been exposed for many generations to the desert atmosphere, have retained the darker pigmentation and other characteristics proper to the coastal plain.

Again, both Grinnell and Taylor[17] have taken specimens of *Peromyscus maniculatus,* which they believe to be typical representatives of the desert race *sonoriensis,*

[15] It is interesting to note that the often cited case of the Porto Santo rabbit has been recently put in quite a new light by the investigations of G. S. Miller, who finds that this transplanted race is merely the *unmodified* rabbit of southern Europe. (Catalogue of the mammals of western Europe, in the British Museum, London, 1912.)

[16] University of California Publications in Zoology, Vol. 10, No. 9, 1913.

[17] University of California Publications in Zoology, Vol. 7, No. 7, 1911.

high up in the mountains of California and Nevada. These points are continuous with the main habitat of the subspecies in the desert lowlands and plateaus, in the sense that no abrupt barriers intervene, but they present very great differences in climate and vegetation.

Facts of this sort—"natural experiments," as we may call them—seem to show that these subspecific differences manifest themselves in a large degree independently of climatic conditions, in other words, that they are of germinal rather than of somatic origin.

But these "natural experiments" are not entirely conclusive, for we can never be quite certain what the actual condition are which Nature has imposed in a given case. Granting that these darker song-sparrows in the desert are actually invaders from the coastal plain, we have no means of knowing how long they have been exposed to the desert conditions. Also is it definitely known that their restricted habitat in certain portions of the desert does not agree with their original habitat in respect to those factors which are really essential in determining their characteristic coloration?

My own first attempt at transplantation consisted in bringing a considerable number of specimens of *P. m. sonoriensis* from the vicinity of Victorville to Berkeley.[18] At the latter point, the mice were kept in cages, freely exposed to the air, and under atmospheric conditions as nearly natural as possible. For control, numbers of the Berkeley race were reared in neighboring cages. The result of this experiment I can state briefly: *Neither the originally introduced animals nor their offspring, nor their grandchildren, have thus far shown any perceptible approach to the local type. They are still obviously of the sonoriensis race.* If there is any tend-

[18] The contrast between the climatic conditions at the two points is indicated, to a certain extent, in the foregoing table. But this does not show one of the most characteristic differences, namely, the relatively enormous diurnal fluctuations of temperature and humidity in the desert, as compared with those in the coastal region.

ency for these mice to be modified in the direction of *gambeli*, this tendency is not sufficiently great to be detected through any mere gross comparison, based upon qualitative characters. Unfortunately, these two races differ significantly in color characters only, so that an exact quantitative test of this question is not here possible. But I am now rearing *P. m. rubidus* at La Jolla, an experiment which would seem to be much more promising of decisive results than the one just described.

Enough has been done, therefore, to prove that the color differences between *sonoriensis* and *gambeli* are at least in a large degree germinal and independent of environmental influences acting during a single lifetime. Whether or not they are *wholly* germinal, and if so, whether they can resist change for an indefinite number of generations, remains to be learned. Suppose that we had a shifting of the mean to the extent of one per cent., or even ten per cent., in the direction of *gambeli*. Amid all the natural variability, we certainly should not be able to detect such a change with the unaided eye.

In interpreting the facts that I have offered as to the relative fixity of these color differences of *Peromyscus*, we should have due regard for various other experiments which show that environmental influences may produce notable changes of coloration in the lifetime of an individual. As especially comparable with these tests of my own, though differing completely in the outcome, may be cited the experiments of Beebe.[19] This author produced a marked increase of pigmentation in the feathers of three species of birds by rearing them in an atmosphere of abnormally high humidity.

Beebe's experiments seem to show that pigmentation may in some cases be altered during a single lifetime by changes of humidity. The generally known facts of geographic distribution show that there is in nature a distinct correlation between pigmentation and humidity. My own experiments show that these geographic differ-

[19] *Zoologica*, N. Y. Zool. Soc., Sept. 25, 1907.

ences of pigmentation are capable of being relatively fixed germinally. Bringing these three sets of facts together, they are, in my opinion, most readily harmonized on the assumption that the direct effects of humidity upon the organism may finally become fixed through heredity.[20] But I grant that this assumption is as yet far from proved.

A study of the effects of captivity forms a new line of inquiry which was hardly considered when the present researches were undertaken. For some reasons, however, this line of investigation now seems quite as promising as the search for climatic effects. In the first place, my comparatively meager results in this field already reveal striking differences between the wild stock and the individuals of the first generation reared in captivity. These differences relate to absolute size (the domesticated generation being smaller), to length of tail and foot (both shorter in the domesticated) and to the length of the femur and the pelvis, which differ in the same direction. In the case of the femur, in particular, the differences are striking, even upon the most casual inspection, and they are absolutely certain statistically. They hold for both sexes and for both of the subspecies which have thus far been tested in this regard. Thus far, no certain effects upon the cranial capacity of the captive lots have been detected.

Such differences as those found probably have nothing to do with the direct effects of external conditions, but are due to the *activities* of the animal. For this reason, they are of much higher value as a test of the Lamarckian principle, which could help us most in explaining the perfecting of active parts through use or of their degeneration through disuse.

The experimental results just referred to suggested the possibility that some of the differences found to occur in nature might have had a functional basis. The larger

[20] Whether this results from ''somatic'' or ''parallel induction'' need not concern us here.

feet and tail of *P. m. rubidus,* for example, might be attributed to its (assumed) greater activity. Unfortunately for this theory, the pelvis and femur of *rubidus* is no greater (slightly smaller, it would seem) than in the other races. Had the enlargement of the foot been due to greater functional activity the skeletal parts named would probably have also undergone an increase in size.

From a series of measurements upon the skeletons of wild and domestic fowls and rabbits, Darwin inferred a relative decrease in the size of the wings of the former and of the cranial capacity of the latter, under the influence of domestication. These conclusions may well be true, but the evidence offered seems to be open to several objections. (1) They are based on too small numbers and lack the precision demanded by modern statistical work. (2) We can only infer the exact nature of the wild stock from which the domesticated races are descended. (3) We can not judge of the extent to which artificial selection may have played a part in bringing about these differences. It was perhaps considerable. (4) We do not know how much of this modification results from use or disuse during the animal's own lifetime. It might be contended that the change was not congenital at all.

Lapicque and Girard have, in the main, demonstrated a smaller cranial capacity in domesticated animals as compared with wild ones, but aside from the more rigorous statistical methods employed by these authors, the biological significance of their results is open to the same objections as I have stated in the case of Darwin's.

Hatai[21] has shown that the albino rat has a smaller brain than the wild Norway rat, when individuals of the same size are compared. This proves nothing definite, however, as to the effects of domestication, a fact which the author recognizes. The deficient brain of the albino may have been part of the same mutation which brought about the albinic condition. Or it may have resulted in part from the selection of tamer individuals. Or the

[21] *Anatomical Record,* Vol. 3, 1909, p. 245.

difference between the two races may be purely ontogenetic. Indeed, Donaldson[22] seems to have demonstrated the effects of exercise upon the weight of the nervous system of the rat.

By taking a known stock of wild mice and measuring each successive generation reared in captivity, and by being careful to avoid selection, it would seem that the foregoing ambiguities of interpretation might in a large degree be obviated. It is possible, therefore, that this phase of the subject deserves quite as much attention as the problems relating to the physical environment and the distribution of subspecies.

Hybridization has thus far failed completely between *rubidus* and *gambeli* (48 matings). The Berkeley *gambeli* has, however, been successfully crossed with *sonoriensis* and some young of an F_2 generation have already been obtained. Owing to the intergrading and widely overlapping character of the differences between these two races, it does not seem likely that they will lend themselves well to Mendelian analysis. But it would be idle for me to discuss the results of these crosses at the present stage of the experiments. Further attempts will, of course, be made to obtain hybrids between the more widely separated races.

(Since writing the foregoing, several successful matings between *rubidus* and *sonoriensis* have been effected.)

[22] *Journal of Comparative Neurology*, Vol. 21, No. 2, 1911.

SHORTER ARTICLES AND DISCUSSION

ADDITIONAL EVIDENCE OF MUTATION IN ŒNOTHERA

In a group of recent papers Bartlett reports on the remarkable behavior of certain wild species of *Œnothera* grown in large cultures, which behavior he regards as strong evidence for the mutation theory of De Vries. The facts are presented very clearly, but there is, however, a point of view which has not been considered in the interpretation of the conditions in his material, certain possibilities that must be reckoned with in the critical examination of such evidence. The suggestions that I shall offer will concern chiefly the genetic purity of the forms studied, a condition which is of course basic to studies on mutation as well as to Mendelian experimentation.

Œnothera pratincola Bartlett[1] is a small-flowered, close-pollinated species apparently common in the North Central States. Seven strains derived from wild mother plants at Lexington, Kentucky, gave rise to a variant, *nummularia,* which differs from the parent type in the form of the seedling leaves, foliage, pubescence of the ovary and calyx, and in the manner in which the calyx is ruptured in the opening of the flower. *Nummularia* appeared with a frequency of about 1 plant to every 400 seeds sown and 1 plant to every 250 seedlings since the germination of the seeds in the earth was 66 per cent. Two of the strains produced *nummularia* in both the F_1 and F_2 generations. Further studies will be undertaken to determine whether *pratincola* will continue to give *nummularia* or whether it may perhaps in later generations produce stable individuals. *Nummularia* develops a low percentage of good pollen (less than 50 per cent.) while *pratincola* has a high proportion (90 per cent.); *nummularia* also forms very few good seeds to a capsule, and of these only 34 per cent. are viable. Small cultures grown from *nummularia* seeds gave no plants of *pratincola,* but certain new forms appeared. The high degree of sterility both gametic and zygotic shown by *nummularia* is striking and demands study, for it will make a difference in the interpretation of the behavior of this plant whether the sterility is physiological or genetic in character.

[1] Bartlett, H. H., "Additional Evidence of Mutation in *Œnothera,*" *Bot. Gaz.,* Vol. LIX, p. 81, 1915.

Cultures of *pratincola* and *nummularia* should be grown from germinations established experimentally to be complete, with records of the residue of sterile seed-like structures, and the two forms should be crossed with the purest *Œnothera* known to determine whether or not the F_1 hybrid generations are uniform. Should F_1 hybrid generations consist of distinct classes we would be justly suspicious of the purity of the stock.

A second paper of Bartlett[2] describes a series of cultures of *Œnothera stenomeres,* a cruciate-flowered species from Montgomery County, Maryland. Two sharply marked new types were produced by the typical form of the species, *gigas* represented by one specimen with 28 chromosomes, and *lasiopetala* with hairy petals. The *gigas* plant appeared in the F_4 generation of a line of *stenomeres.* The peculiarities separating it from *stenomeres* are similar to the distinctions between *Lamarckiana* and its derivative *gigas.* Thus both *gigas* forms are more persistently biennial in habit than their parents, both have thicker, broader leaves, stouter stems, larger buds, thicker fruits, 4-lobed pollen grains, and twice as many chromosomes. A progeny of 63 individuals from the original *gigas* plant consisted of 54 typical *gigas,* 6 narrow-leaved variants, and 3 "secondary mutations"; the form thus, as with the *gigas* from *Lamarckiana,* produces a varied offspring. Two of the "secondary mutations" were dwarfs and one had the characters of *lasiopetala,* hairy petals, and in addition certain of the stamens were also hairy.

Lasiopetala was noted in an F_3 generation and also in two cultures of an F_4; it is of infrequent occurrence, only 5 plants in all being observed. The plants formed persistent rosettes (*stenomeres* being annual) and only one branch produced flowers, these with hairy petals. The pollen of *lasiopetala* is 40–50 per cent. perfect; that of *stenomeres* 60–80 per cent. An F_1 progeny of 116 plants from selfed *lasiopetala* gave 60 per cent. typical *stenomeres* and 40 per cent. *lasiopetala,* thus behaving like *Œnothera lata* and *O. scintillans* in throwing their parent form *Lamarckiana.*

Of these two new types derived from *O. stenomeres* the *gigas* plant is remarkable as being another of the very few œnotheras discovered with the quadruploid number of chromosomes (28); triploid forms usually named *semigigas* have been described from a number of lines. The hairiness of the petals in *lasiopetala* is

[2] Bartlett, H. H., "The Mutations of *Œnothera stenomeres,*" *Amer. Jour. Bot.,* Vol. II, p. 100, 1915.

regarded by Bartlett as a character new to the genus. Bartlett emphasizes the fact that the characters of neither type could be interpreted as the result of segregation following hybridization, which may be true, but I do not think from this that it follows that neither type can be the result of hybridization. I am not willing to admit that hybrids present only combinations of characters derived from their parent lines. It seems to me reasonable to believe that in hybrids at times the interaction of elements modifies the old or produces new factors. The species *stenomeres* and the derivatives *gigas* and *lasiopetala* have not been tested for genetic purity by cross-breeding with relatively stable types and the problems of gametic and zygotic sterility have not yet been attacked.

The final paper of this group of Bartlett's[3] deals with an extremely interesting situation developed in cultures of *Œnothera Reynoldsii* from Knoxville, Tennessee. This is also a small-flowered, close-pollinated species, and its peculiarity lies in an ability to throw extraordinarily large classes of dwarfs. There are two types of dwarfs: (1) *semialta* somewhat smaller than the typical *Reynoldsii* and intermediate between it and the smaller dwarf; (2) *debilis*. A plant of *Reynoldsii* in the F_3 produced 29 individuals like itself, 32 plants of *semialta*, and 18 of *debilis*, *i. e.*, 60 per cent. of its offspring were dwarfs. *Semialta* throws *debilis*, but no *Reynoldsii*. *Debilis* apparently can produce no *Reynoldsii* or *semialta* and breeds true except for an occasional variant *bilonga* which was also found in one culture from typical *Reynoldsii*.

With respect to the dwarfs we have here presented a beautiful series leading from the unstable parent type *Reynoldsii* through the more stable *semialta* to the most stable and most extreme dwarf *debilis*. Bartlett calls the behavior mutation *en masse*, but confesses that it bears a certain degree of resemblance to Mendelian segregation. We should very much like to see this study repeated on a larger scale and with experimental germination of the seed so that we may be sure of the ratios and also certain that the cultures have given us all of their possible progeny. Segregation *en masse* seems to the writer likely to be a more probable explanation of the phenomena than mutation.

The form *bilonga* derived from the dwarf *debilis* offers a particularly interesting problem. It is similar to *semialta* except that

[3] Bartlett, H. H., "Mutation *en masse*," AMER. NAT., Vol. XLIX, p. 129, 1915.

the fruits are twice as long. The capsules sometimes reach the length of 70 mm. and average above 60 mm.; they are very much the longest fruits reported in the subgenus *Onagra*. Bartlett regards this large size of capsule as the origin of a new character. Now capsule size obviously depends upon the number of ovules produced which develop into seeds. It thus becomes an important matter to obtain the data on ovule sterility in the species *Reynoldsii* and the derived forms *semialta, debilis* and *bilonga*. Ovule sterility is widespread among the Œnotheras as all students of the genus know. Should it be found that *bilonga* produces a very much greater number of ovules than *debilis* and the other types this fact would indicate a true progressive advance. It may, however, be found that the smaller size of the capsules of *Reynoldsii semialta,* and *debilis* is due to ovule sterility, *i. e.*, to the inability of a large proportion of the ovules to set seed. This would point to a very different interpretation of the conditions in *bilonga,* and might indicate that *bilonga* is an example of reversion towards an ancestral type in which a large capsule was correlated with a high degree of ovule fertility.

In the comments which I have presented on the extremely interesting facts discovered by Bartlett no attempts have been made to offer exact explanations in line with Mendelian analysis. It is not difficult to spin hypotheses on assumptions which have been neither established nor disproved, but such creations are hardly worth the effort when the facts are within grasp. My main point is a constant questioning of the genetic purity of the material with which Bartlett has worked from the standpoint developed in my forthcoming paper "The Test of a Pure Species of *Œnothera*."[4] It is impossible to discuss this subject in the short space of a review. The most important test is that of cross-breeding with the purest species known, to judge from the uniformity of the F_1 hybrid generation whether or not the parent types are pure. I also firmly believe that all exact genetical work on œnotheras must make use of methods of experimental germination to ensure complete progenies from the viable seeds and tc permit the preservation of a residue of ungerminated structures that may be examined.[5] There is in addition the determination

[4] To appear in the *Proceedings of the American Philosophical Society*, Vol. LIV, 1915.

[5] Davis, B. M., "A Method of Obtaining Complete Germination of Seeds in *Œnothera* and of Recording the Residue of Sterile Seed-like Structures," *Proc. Nat. Acad. Sci.*, Vol. I, p. 360, 1915.

of degrees of sterility both gametic and zygotic, and the consideration of whether such sterility is genetic or physiological. From such tests it is possible to reach much clearer conclusions on the genetic purity of *Œnothera* material than has been possible in the past.

Finally reference should be made to the important confirmation by De Vries[6] of the studies of Stomps on *Œnothera biennis* L. In large cultures totaling 8,500 plants from Stomps's selfed line De Vries obtained 8 plants of a dwarf *biennis nanella* about 0.1 per cent., 4 plants of *biennis semigigas* (21 chromosomes) about 0.05 per cent., and 27 plants of the color variety *biennis sulfurea* about 0.3 per cent. Since the percentages from *Lamarckiana* are for *nanella* 1–2 per cent. and for *semigigas* 3 per cent., it would appear that *biennis* is the more stable of the two species, although the color variety *biennis sulfurea* is a new type in experimental studies in *Œnothera.* A culture of over 1,000 plants from selfed seed of *biennis sulfurea,* all with pale yellow flowers, produced 2 dwarfs, thus giving what De Vries calls a "double mutant," *O. biennis* mut. *sulfurea* mut. *nanella.*

This behavior of *Œnothera biennis* is to the writer much more trustworthy evidence for mutation than that presented from the studies on *Lamarckiana* since *biennis* has a record of a long history as a species on the sand hills of Holland, where there appears to have been little probability of recent contamination. However, the showing of "mutants" from *biennis* does not appear very encouraging for the mutation theory of organic evolution when it is remembered that *biennis nanella* is frequently weakly or diseased, that *biennis semigigas* is self sterile, and that *biennis sulfurea* appears to be a retrogressive form having lost the power of producing normal yellow flowers. Although the Dutch *biennis* of all the œnotheras so far brought into the experimental garden still seems to me the form most free from suspicion of genetic impurity, nevertheless, the line of Stomps's has not, so far as we know, been subjected to all of the tests of a pure species. Until these tests are made it is not safe to assume that this material is wholly pure. It seems to me not improbable that other species of *Œnothera* will eventually be isolated more stable than the Dutch *biennis.*

BRADLEY MOORE DAVIS

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June, 1915

[6] De Vries, Hugo, "The Coefficient of Mutation in *Œnothera biennis* L.," *Bot. Gaz.,* Vol. LIX, p. 169, 1915.

THE VALUE OF INTER-ANNUAL CORRELATIONS

If $x_1, x_2, x_3 \cdots x_n$ be measures taken on the n individuals of a series in a given year and $x_1', x_2', x_3' \cdots x_n'$ be similar measures taken in a subsequent year, the correlation between the first and second measures on the same individual $r_{xx'}$, may be designated as a direct inter-annual correlation.[1] The purpose of this review is to illustrate the usefulness of such constants, with a view to extending their application, by bringing together examples of inter-annual correlations from various fields.

The immediate value of such coefficients may be purely scientific, economic, or both theoretical and practical.

Practically such means of prediction as correlation and regression formulæ should find wide application in breeding operations where it is desirable to weed out or send to the butcher at the earliest possible moment those individuals which can not be kept with the maximum profit. If the correlation between the egg production of a fowl in her pullet year and her laying capacity in any subsequent year be high, it is clear that those which on the average are to prove unprofitable may be sent to the pot when most desirable for that purpose, and before they have consumed two or more years' feed without yielding the maximum return in eggs. If, on the contrary, there be no correlation, the labor of selection in the pullet year is an unnecessary expense. If a cow's milking capacity be closely correlated with her milking record in her heifer year, the culling of dairy herds may be profitably carried out in the first year. In plant breeding experiments, involving either sexual or vegetative reproduction, selection of individuals for future propagation must be made, and at as early a date as possible. If the future yield per plant of hay can be estimated with considerable accuracy from a first year's culture the process of selecting clonal strains can be carried out with far greater rapidity than if one must wait for the results of subsequent years' tests. In all such cases the finality of a first judgment must depend in large degree upon the closeness of correlation between the results of successive experiments—in short upon the value of the inter-annual correlation coefficient.

[1] Cross inter-annual correlations in which the measures taken are of a different sort are sometimes useful, but examples of such are not considered in this review.

In dealing with egg production Pearl and Surface[2] give as the correlation in number of eggs for first and second year

$$r = .032 \pm .083,$$

a value which, though positive, is clearly insignificant with regard to its probable error.

Thus in this particular case the performance of the first year furnishes no clue to that of the second. With respect to egg-laying capacity, the record of the pullet year furnishes no criterion for elimination from the flock.

For milk yield in cattle the case seems to be quite different. Gavin[3] has found that there is a medium correlation between (*a*) the "revised maximum"[4] yield in quarts of successive lactations, and (*b*) between the "revised maximum" of the individual lactation periods and the highest revised maximum reached by the animal.

So far as I am aware the only worker who has published correlations between the characters of the same plant individuals in different years is Clark[5] whose results have been noted in these pages by Pearl.[6]

The correlation tables and constants show that plants of a given class in any year (height or weight of hay produced) will be highly variable in a subsequent year, but will on the average deviate from the mean of the whole culture of the year in the same direction and to about half the extent of the type selected in the preceding year. Thus if selection were made on the basis of a single year's test only, many individual plants of low yield would be discarded which in a subsequent year would have taken higher rank, while high-yielding plants would be retained which subsequently would give disappointing results. On the whole, however, the yield of a hay plant one year does furnish a valuable index to its yield in a subsequent year.

[2] Pearl, R. and F. M. Surface, "A Biometrical Study of Egg Production in the Domestic Fowl," I, *Bull. Bu. Anim. Ind.*, 110, 66, 1909.

[3] Gavin, Wm., "Studies in Milk Records: On the Accuracy of Estimating a Cow's Milking Capacity by Her First Lactation Period." *Jour. Agr. Sci.*, *5*, 377–390, 1913.

[4] "Revised Maximum" milk yield is the maximum day yield which is three times reached or exceeded in a lactation.

[5] Clark, C. F., "Variation and Correlation in Timothy," *Bull. Cornell Agr. Exp. Sta.*, 279, 1910.

[6] Pearl, R., AMER. NAT., *45*, 418–419, 1911.

That we are dealing with a real measure of the relatively permanent differentiation of individuals, and not with merely temporary differences due to growth, is indicated by the fact that the correlations between a first and a third year are about the same as those between a first and a second or a second and a third.

In other fields of plant industry such methods may be profitably applied. For example Sievers[7] after discussing at some length the question of the differentiation of belladonna plants with respect to alkaloidal content, warns the reader that "the investigation has hardly progressed far enough to yield any definite conclusions" but says in summarizing his data:

> A considerable number of plants with leaves rich in alkaloids in one season are found to have equally rich leaves in the following season. Furthermore, they frequently manifest the same characteristics at the various stages of growth during the season in comparison with other plants. The same facts are true with regard to plants which bear leaves with a low percentage of alkaloids.

How much more definite is the information conveyed by the simple statement that the inter-annual correlation[8] between the alkaloidal content for 1911 and 1912 is

$$r = .513 \pm .066!$$

Such studies as those by Stockberger on individual performance in hops[9] may be facilitated by the use of inter-annual correlation coefficients. He gives only the extremes of his series of individuals, but from these the correlations between yield per hill for different years are:

	Lowest Hills	Highest Hills
1909 and 1910	$.29 \pm .17$	$.59 \pm .13$
1910 and 1911	$.55 \pm .13$	$.52 \pm .14$
1909 and 1911	$.43 \pm .15$	$.30 \pm .18$

Such constants, deduced from materials which almost certainly

[7] Sievers, A. F., "Individual Variation in the Alkaloidal Content of Belladonna Plants," *Jour. Agr. Res.*, *1*, 129–146, 1913.

[8] In computing this coefficient a number of inconsistencies in the data table were discovered. The constant as given is probably as nearly correct as can be found from the available data.

[9] Stockberger, W. W., "A Study of Individual Performance in Hops," *Proc. Amer. Breed. Ass.*, *7*, 452–457, 1912.

do not show the full strength of the correlation, remove at once all question concerning the relatively permanent differences in productiveness of the individual hills.

Consider next an illustration from hybridization of measurable characters.

Goodspeed and Clauson[10] have given the mean values of measurements of the flowers of individual plants of *Nicotiana* hybrids cultivated in 1912 and of corollas of the same plants cut back and flowered in 1913. The correlations between the mean dimensions for the two years I find to be:

N. Tabacum var. *macrophylia* ♀ × *N. sylvestris* ♂
F_1 plants, $N = 21$.
For spread of corolla, $r = .044 \pm .147$.
For length of corolla, $r = .169 \pm .143$.

Hybrid produced by crossing F_1 of the hybrid *N. Tabacum* "Maryland" ♀ by *N. Tabacum* ♂, with *N. sylvestris*, $N = 19$.
For spread of corolla, $r = .560 \pm .106$.
For length of corolla, $r = .788 \pm .059$.

These correlations show at once the high degree of uniformity of the F_1 of the first as compared with that of the second series. In all four cases the signs of the coefficients are positive, but those of the first class are insignificant in comparison with their probable errors. In both cases length of corolla is more closely correlated than breadth. Possibly this is due to errors of sampling only, or to greater difficulty in obtaining an exact measure of the spread of the limb. It may, however, indicate that some characters are more sharply and permanently differentiated from individual to individual than others.

That the latter may sometimes be the case is clearly shown by unpublished data of my own for the ligneous perennials *Staphylea trifolia* and *Hibiscus Syriacus*.[11]

[10] Goodspeed, J. H., and R. E. Clauson, "Factors Influencing Flower Size in *Nicotiana* with Special Reference to Questions of Inheritance," *Amer. Jour. Bot.*, *2*, 232–274, 1915.

[11] The constants are based in all cases on mean values of the characters of ovaries of shrubs well established in the Missouri Botanical Garden. In such work the number of individuals can never for practical reasons be very large, if a fairly large number of countings be made for each shrub. Furthermore much of the work which one does may be lost by some accident which precludes the securing of countings from each individual every year. If an individual is not represented in both of a pair of years it must be omitted entirely.

The accompanying tables show the correlations deduced for the characters indicated.[12]

INTER-ANNUAL CORRELATIONS FOR FRUITS OF STAPHYLEA

Relationship	Correlation for 1906 and 1908, $n = 19$	Correlation for 1907 and 1908, $n = 15$	Correlation for 1908 and 1909, $n = 20$
Ovules and ovules	.445 ± .069	.816 ± .058	.872 ± .036
Seeds and seeds	.063 ± .154	.064 ± .173	.056 ± .150
Asymmetry and asymmetry	.748 ± .068	.102 ± .172	.205 ± .145
Locular composition and locular composition	.601 ± .099	.294 ± .159	.335 ± .134

INTER-ANNUAL CORRELATION FOR FRUITS OF HIBISCUS, $n = 23$.

Relationship	Correlation for 1907 and 1908
Sepals and sepals	.451 ± .112
Bracts and bracts	.836 ± .042
Ovules and ovules	.941 ± .016
Seeds and seeds	.630 ± .085
Asymmetry and asymmetry	.747 ± .062
Locular composition and locular composition	.725 ± .067
Fertility and fertility	.610 ± .088
Correlation and correlation	.035 ± .141

The constants are very irregular in magnitude, but are without exception positive in sign. In many instances they are large. Thus in these individual shrubs which taxonomically show no differences[13] there is nevertheless a distinct differentiation in respect of the great majority of the characters examined.

While the probable errors are large the evidence warrants the conclusion that some are decidedly more highly correlated than others.

[12] Sepals = mean number of sepals in calyx.

Bracts = mean number of bracts in involucre.

Ovules = mean number of ovules formed per fruit.

Seeds = mean number of seeds matured per fruit.

Asymmetry = average radial asymmetry in the distribution of the number of ovules per locule. For method of computation see *Biometrika*, Vol. VII, pp. 477–478, 1910, and AMER. NAT., Vol. XLVI, p. 480, 1912.

Locular composition = average number of locules per fruit with an odd number of ovules. See citations above.

Fertility = coefficient of fertility (mean seeds per fruit) (mean ovules per fruit).

Correlation = coefficient of correlation between number of ovules and number of seeds per locule.

[13] I believe one of the *Hibiscus* shrubs had lighter flowers than the rest.

In *Hibiscus* the differentiation of the individuals with respect to number of bracts seems to be greater than that for number of sepals. For both *Staphylea* and *Hibiscus* the correlation for ovules is generally high. It is in every instance higher than that for mean number of seeds matured per fruit. Correlation for both mean number of seeds per fruit and relative number of seeds matured has a moderately large value in *Hibiscus*, but in *Staphylea* it is sensibly 0. In both species such characteristics of the ovary as radial asymmetry and locular composition seem to be rather sharply differentiated from individual to individual. This is probably due in part to differentiation with respect of number of ovules per fruit, but further discussion of the problem would be out of place in a note, the only purpose of which is to call attention to the usefulness, in both applied and pure science, of a quantitative means of detecting and expressing permanent differentiation.

In this brief review I have made no attempt to discuss fully all the biological phases of the problems suggested. The analysis of the data may in several instances be carried much further by the use of the statistical tools. Perhaps enough has been said to indicate that inter-annual coefficients may be of real service in practical animal husbandry, in plant breeding and in morphology and physiology. More than usefulness is not to be expected of any method.

J. Arthur Harris

THE PHENOMENON OF SELF STERILITY

In my paper which appeared in The American Naturalist, Vol. XLIX, p. 79, the last seven lines on page seventy-nine should read as follows:

> Self-sterile plants crossed with self-sterile plants gave only self-sterile offspring. Certain self-fertile plants, however, gave only self-fertile offspring either when self-pollinated or when crossed with self-sterile plants. Other self-fertile plants gave ratios of 3 self-fertile to 1 self-sterile offspring when self-pollinated, and ratios of 1:1 when crossed with pollen from self-sterile, etc.

E. M. East.

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SOME EXPERIMENTS IN MASS SELECTION

PROFESSOR W. E. CASTLE

BUSSEY INSTITUTION, HARVARD UNIVERSITY

AT the close of an interesting review of "seventeen years selection" of the character winter egg production in Barred Plymouth Rock fowls, made at the Maine Agricultural Experiment Station,[1] Dr. Pearl compares his results with those of Phillips and myself[2] in selecting for a like number of generations the hooded pattern of rats and concludes that the same interpretation should be given to both series of experiments, viz., that selection can change a population but not a character.

Without discussing for the moment the validity of the now world-famous generalization of Johannsen, which Pearl here accepts for his fowls and seeks to extend to our rats, I wish to point out some differences between the two cases which make a direct comparison between them difficult and conclusions based upon them of unequal validity.

The character winter egg production in fowls is on Pearl's showing extremely difficult to determine. It is necessarily an unknown quantity in all male birds, which themselves produce no eggs, and any influence which

[1] "Seventeen Years Selection of a Character Showing Sex-linked Mendelian Inheritance," AMERICAN NATURALIST, Vol. 49, pp. 595–608, 1915.

[2] "Piebald Rats and Selection," Publ. No. 195, Carnegie Institution of Washington, 1914.

males may exert on the egg-production of their daughters can be tested only by an indirect and rather uncertain process. Only in the case of females is the character directly measurable and then only for such females as (1) are hatched "after April 1 and before June 1," (2) survive all the accidents of chickhood and adolescence, (3) escape all attacks of disease and are kept continuously free from parasites, and (4) are properly fed and housed. For any bird which dies, is disabled or becomes seriously ill under ten months old, the character is an unknown quantity. These limitations make the proportion of birds which can be accurately rated as regards the character extremely small, and reduce correspondingly the material on which selection can be practised.

Contrast with this situation that regarding the hooded pattern of rats. This character is possessed by every individual of both sexes and is inherited equally through either sex. The character is fully developed in its final form within a week after birth, months before sexual maturity is attained. This makes it possible to grade the animals accurately while they are still very young and to discard at once all individuals which fall below the adopted standard. Selection thus has a vastly greater amount of material to work with, and the variation in each generation can be ascertained with a completeness and accuracy quite impossible in the case of winter egg production in fowls.

It is scarcely necessary to point out that upon the completeness of one's knowledge of the character and extent of variation depends his ability to take advantage of that variation by systematic selection. By this criterion winter egg production is very poor material on which to base an experimental test of "mass selection," whereas the hooded pattern of rats is material admirably adapted for the purpose. Many times has the fact been commented upon that Mendel's fortunate choice of peas as material for his studies of hybridization was largely responsible for his success where others failed. If one wishes to test

a theory he must choose material suited to the purpose. No adequate test of the efficacy of mass selection can be obtained from material which can not be accurately judged in the mass.

Pearl points out further limitations of his material in the statement "that phænotypic variation of the character fecundity, in fowls, markedly transcends, in extent and degree, genotypic variation." That is, non-heritable causes of fecundity are in excess of heritable causes and serve to obscure the occurrence of the latter. Further, Pearl says:

> It is quite impossible in the great majority of cases to determine with precision what is a hen's genetic constitution with respect to fecundity from an examination of her egg record alone.

If then one has reared his pullets to the age of one year, has kept them free from disease and parasites, has fed and housed them properly and has even trap-nested them and recorded their eggs all winter, still he has no sufficient basis on which to base a selection. He must first rear and test their progeny in the same way. Pearl's statements on this point, the accuracy of which I do not question, are sufficient to show the entire unsuitability of his material for testing the efficacy of mass selection.

One might with propriety even question whether such a thing as inherited capacity for winter egg production exists in fowls, but on this point, I think, another investigation[3] made by Pearl is conclusive, in which he crossed Cornish Indian game fowls, which are poor winter layers, with Barred Plymouth Rocks which are fairly good winter layers. Reciprocal crosses were made in both of which the daughters showed resemblance to the racial winter egg productiveness of the sire's race. This result indicates that a sex-linked genetic factor of some sort exists which affects winter egg production in fowls. But since the fecundity of the offspring was obviously influenced by the mothers' race as well as by the father's race, Pearl was

[3] "The Mode of Inheritance of Fecundity in the Domestic Fowl," *Jour. Exp. Zool.*, Vol. 13, p. 153, 1912.

led to suggest the existence of a second fecundity factor which was *not* sex-linked. He assumes that this second factor, like the first, is a Mendelizing factor, but without any sufficient published evidence for either conclusion. To this I called Dr. Pearl's attention soon after the publication of his paper and suggested that if possible the data be put on record in such form as to allow of testing this and other hypotheses concerning the genetic factors concerned. For one-factor, two-factor, ten-factor and infinity-factor Mendelian hypotheses would call for very different ratios and distributions of fecundity among the offspring. He replied that the data could not be so given without an amount of work which he considered unprofitable. We are left, therefore, with only this information concerning Pearl's pullets, whether each one laid *more* or *less* than 30 eggs in its first winter. If we knew *what* number each one laid, we might form an intelligent opinion as to whether Mendelian factors are involved, and if so how many, in the same way that we can test Mendel's conclusions concerning the independent inheritance of yellow cotyledon color and round seed form in peas because he tells us the actual proportions of the various sorts of peas reported for each plant. Being denied such information by Pearl, it is useless to discuss his two-factor hypothesis, for its correctness can be neither proved nor disproved.

Leaving aside the question whether any inherited *factor* has changed as a result of selection in Pearl's experiments, which we have no means of investigating, we can consider only the question whether the gross winter egg production has changed. As a basis for judgment he gives us the averages of winter egg production year by year for sixteen years. Pearl's graphic presentation of the data (assuming that the considerable fluctuation recorded is not significant) indicates a steady decline of the general flock average during the first nine years of the experiment and a steady recovery and further increase during the next seven years, which he ascribes to

the different basis of selection in the two periods. But it is hard to believe that this entirely explains the difference in result. One notices for example that during the period of ostensible decline the highest average fecundity (45.23) is recorded when the number of birds under observation is smallest (48) and the lowest average (19.93) is recorded when the flock is largest (780). Further, in the later seven-year period of "improvement," the number of birds tested declines as their average fecundity rises. Has not the better environment and lessened competition of small numbers possibly something to do with the changes noted? Is it certain that genetic agencies are responsible for the differences observed? Pearl himself nowhere states that the selection practised during the earlier period had produced positive deterioration; he merely states that "there was no change of the mean in the directon of the selection" during this period when selection was based on high production without progeny tests. But as soon as progeny tests were made an additional feature of the basis for selection Pearl notes immediate results, viz., the immediate isolation of a strain which in its first year made a record for high productiveness only once equalled in the six subsequent years. How many successive selections were made in this period, we are not informed, but since it would require at least two years to make a combined performance and progeny test, it would seem that not more than three successive selections can have been carried out on this basis in the seven year period from 1908 to 1915. It may fairly be questioned whether this is an adequate test of the effectiveness of mass selection. The total number of individuals tested during this period is, according to Pearl's table, 1,655. For the entire seventeen years of selection it is 4,842.

The total number of animals graded in our selection experiments with rats heretofore published is 20,645, and the number of generations involved 13. Since those figures were compiled, four additional generations of

rats have been reared in the straight selection series, bringing the total number of animals observed in this experiment up to 33,249, and the total number of generations of selections up to 17, numbers certainly more nearly justifying the term "mass selection" than those studied by Pearl. As no previous account of this experiment has been given to readers of the Naturalist, a brief review of its salient features may be appropriate here.

Experiments made by MacCurdy and by Doncaster had shown that the hooded pattern of rats is a Mendelian recessive character dominated in crosses by the "self" or entirely pigmented condition of wild rats and of certain tame races. The F_2 ratio obtained in crosses between hooded and self rats is an unmistakable monohybrid ratio, viz., 493 hooded: 1,483 self, or 24.9 per cent. hooded. The hooded pattern is subject to slight fluctuations in the relative amounts of pigmented and unpigmented surfaces, and though these slight plus and minus variations are such as are usually disregarded in Mendelian analyses, MacCurdy's investigations had indicated that they are to some extent inherited. It was our purpose in starting the selection experiments to ascertain whether the observed fluctuations were capable of increase and summation through the action of repeated selection, a possibility denied for all such cases by de Vries and Johannsen on theoretical grounds and quite incompatible with notions prevailing then as to the "gametic purity" of recessives. This "pure line" idea Pearl still maintains on the basis of his observations of the winter productiveness of his pullets. But, as I have tried to show, his material is no more adequate than that of Johannsen, which involved no demonstrated Mendelian character whatever. For, though Pearl *assumes* that winter egg productiveness of fowls involves a "sex-linked Mendelian character" he has withheld from publication the only facts on which such an assumption may legitimately be based.

Our selection experiments with hooded rats began in

1907. The initial stock consisted of less than a dozen individuals all "pure recessives," which produced only "recessive" hooded young, in accordance with Mendelian expectation. But though all the young were recessive (hooded), all were not exactly alike, and to assist in their classification we devised arbitrary "grades" of increased (plus) or decreased (minus) pigmentation as compared with the *modal* (zero) condition in our hooded race. The scale of "grades" is shown in part in Fig. 1. It has

FIG. 1. Arbitrary set of grades used in classifying the fluctuating variations of hooded rats.

been found necessary to extend it in both directions, beyond the range shown in the figure, in order to admit the new grades of rats which have made their appearance as the experiment progressed. The first plus-selected parents produced 150 offspring ranging in grade from + 1 to + 3, mean + 2.51. The first *minus-selected* parents produced 55 offspring ranging in grade from − 2 to + $\frac{1}{4}$, mean —1.46. It will be observed that the ranges of the young produced in the two selections were practically continuous with each other, though they did not actually overlap. But actual overlapping did occur in the following generation, in which no advance was made in the mean grade of the parents, practically all the available females being used as parents in an effort to increase the stock. The grade of the offspring also remained practically stationary in this second generation (see Tables I

TABLE I

RESULTS OF THE PLUS SELECTION OF HOODED RATS CONTINUED THROUGH SIXTEEN SUCCESSIVE GENERATIONS

Generation	Mean Grade of Parents	Mean Grade of Offspring	Lowest Grade of Offspring	Highest Grade of Offspring	Standard Deviation of Offspring	Number of Offspring
1	2.51	2.05	+1.00	+3.00	.54	150
2	2.52	1.92	−1.00	+3.75	.73	471
3	2.73	2.51	+ .75	+4.00	.53	341
4	3.09	2.73	+ .75	+3.75	.47	444
5	3.33	2.90	+ .75	+4.25	.50	610
6	3.52	3.11	+1.50	+4.50	.49	861
7	3.56	3.20	+1.50	+4.75	.55	1,077
8	3.75	3.48	+1.75	+4.50	.44	1,408
9	3.78	3.54	+1.75	+4.50	.35	1,322
10	3.88	3.73	+2.25	+5.00	.36	776
11	3.98	3.78	+2.75	+5.00	.29	697
12	4.10	3.92	+2.25	+5.25	.31	682
13	4.13	3.94	+2.75	+5.25	.34	529
14	4.14	4.01	+2.75	+5.50	.34	1,359
15	4.38	4.07	+2.50	+5.50	.29	3,690
16	4.45	4.13	+3.25	+5.87	.29	1,690
						16,107

TABLE II

RESULTS OF THE MINUS SELECTION OF HOODED RATS CONTINUED THROUGH SEVENTEEN SUCCESSIVE GENERATIONS

Generation	Mean Grade of Parents	Mean Grade of Offspring	Lowest Grade of Offspring	Highest Grade of Offspring	Standard Deviation of Offspring	Number of Offspring
1	−1.46	−1.00	+ .25	−2.00	.51	55
2	−1.41	−1.07	+ .50	−2.00	.49	132
3	−1.56	−1.18	0	−2.00	.48	195
4	−1.69	−1.28	+ .50	−2.25	.46	329
5	−1.73	−1.41	0	−2.50	.50	701
6	−1.86	−1.56	0	−2.50	.44	1,252
7	−2.01	−1.73	0	−2.75	.35	1,680
8	−2.05	−1.80	0	−2.75	.28	1,726
9	−2.11	−1.92	− .50	−2.75	.28	1,591
10	−2.18	−2.01	−1.00	−3.25	.24	1,451
11	−2.30	−2.15	−1.00	−3.50	.35	984
12	−2.44	−2.23	−1.00	−3.50	.37	1,037
13	−2.48	−2.39	−1.75	−3.50	.34	1,006
14	−2.64	−2.48	−1.00	−3.50	.30	717
15	−2.65	−2.54	−1.75	−3.50	.29	1,438
16	−2.79	−2.63	−1.00	−4.00	.27	1,980
17	−2.86	−2.70	−1.75	−4.25	.28	868
						17,142

and II). In the third and all subsequent generations selection was made as rigorous as possible consistent with the maintenance of a strong colony from which to make further selections. Following each selection an advance in the average grade of the offspring took place attended by a steady movement in the direction of the selection on the part of both the upper and the lower limits of variation. The sixteenth plus selection produced 1,690 offspring (a larger number of individuals than is contained in Pearl's entire seven-year series) *every one of which fell beyond the original range of variation,* which was from $+1$ to $+3$ in the first plus selected generation and from $+3\frac{1}{4}$ to $+5\frac{7}{8}$ in the sixteenth generation. What this change signifies will be better appreciated when I state that $+6$ in our grades is a wholly pigmented or "self" rat, and that the extreme variation noted, $+5\frac{7}{8}$, signifies a rat wholly pigmented except for a few white hairs between the front legs. The *whole race* has accordingly been changed so that *no individual* is longer produced which falls within the original range of variation. Not a dozen rats in this entire generation would be allowed by a fancier in the category of "hooded" rats.

In the minus selection series the results secured are scarcely less striking. Only a very few individuals of the 1,980 sixteenth generation rats, or the 868 seventeenth generation rats fell within the original range of variation, which in generations 1–3 went no farther than grade -2. In all other individuals of the sixteenth and seventeenth generations the "hood" was reduced to an extent never seen in the hooded rats of the fancier, the white areas having covered the neck and in extreme cases the forehead also, leaving only the nose and a patch round the eyes and ears still pigmented.

Pearl (p. 607) commenting on the results of his selections states that he had no reason to think that at the close of the series any individual had been produced superior in productiveness to those which occurred at the outset, but that he had merely secured *more of them,* thus raising

the average. With the rats, however, a very different condition exists. The average is not changed by increase of high-grade individuals merely or chiefly. At the present time *every individual* in the plus selection series and *nearly* every individual in the minus selection series is of higher grade (plus or minus respectively) than *any* individual in the race at the outset. It is not a fallacious change of averages which has taken place; a genuine and permanent racial change has occurred, following step by step upon repeated selection. Generation by generation new grades of offspring have come into existence, more extreme in character than any which existed before, and simultaneously with the advance of the outer limit of variation the inner limit has receded. No great change in variability has attended the selection. The standard deviation has decreased somewhat to about three fifths of its original amount, but has scarcely altered in the last eight or ten generations (see Tables I and II). Rather there has occurred a change in the *modal condition* of the character, about which fluctuation continues very much as before. When the position of the mode changes, as a result of selection, the position of the average and of the upper and lower limits of variation change with it. In a word the *character* changes.

In our 1914 publication Phillips and I were conservative about asserting a change in the single Mendelian unit-character manifestly involved in the hooded pattern. We suggested the possibility that other as yet undiscovered factors might be responsible for the apparent changes observed and awaited the result of experiments then in progress to show whether such a possibility was admissible. I have no hesitation now in saying that it is not. All the evidence we have thus far obtained indicates that outside modifiers will not account for the changes observed in the hooded pattern, itself a clear Mendelian unit. We are forced to conclude that this unit itself changes under repeated selection *in the direction of the selection;* sometimes abruptly, as in the case of our "mu-

tant'' race, a highly stable plus variation; but much oftener gradually, as has occurred continuously in both the plus and the minus selection series. The permanency of these cumulative changes we have tested by repeated crossing of both selected races with the same wild race. The first cross seems to undo to a slight extent the work of selection, causing regression in both plus and minus selected races, but a second back cross with the wild race causes no further regression. Thus, plus-selected rats of mean grade 3.45 were crossed with wild rats and the recessive character was recovered in F_2 in 75 individuals, 24 per cent. of the entire generation. These 75 extracted hooded rats were of mean grade 2.89, a regression of .56 on the mean grade of their hooded grandparents, which is about double the regression shown by the plus selected race when not crossed with wild rats. It seems proper therefore to attribute to the wild cross a part of the regression observed in this case and this I have expressed by saying that crossing the selected race with wild rats tends to *undo* the work of selection. The suggestion was tentatively adopted by Phillips and myself that this *undoing* consisted in the removal of ''modifiers'' of some sort, possibly independent Mendelizing factors. If this explanation were correct, further crossing with wild rats should tend still further to ''undo'' the work of selection, so that ultimately the extracted hooded race should return completely to its original modal state, the zero grade. To test this matter, extracted hooded rats ranging from grade +2 to +4 (mean grade 3.01) were crossed back a second time with pure wild rats. The theory of independent modifiers would lead one to expect further regression as a result of this cross, but no regression was this time observed. Instead an advance of .32 took place bringing the mean of the twice extracted hooded recessives back to about the grade of the uncrossed race. The mean grade of the once-extracted grandparents, loaded in proportion to the number of their twice-extracted hooded grandchildren, was 3.01; the mean of the 263 hooded grandchildren was 3.33.

The number of these grandchildren is large enough to leave no doubt as to the conclusion that no further regression attended extraction of the hooded character a second time from the wild cross. The proportion of hooded individuals to non-hooded is also an unmistakable monohybrid ratio, viz., 263 hooded to 759 non-hooded, or 25.7 per cent. hooded in a total of 1,022 individuals.

This result indicates clearly the untenable character of our provisional hypothesis to explain the altered grade of hooded rats under selection and crossing, by invoking the action of independent modifying Mendelian factors. No evidence is forthcoming from further and more extensive experiments that such modifying factors are concerned in the result. It seems rather that the hooded character, which is a mosaic or balanced condition of pigmented and unpigmented areas, is slightly unstable. It oscillates regularly about a mean condition or grade, these oscillations being not phenotypic merely but in part genotypic so that selection brought to bear upon them is immediately and continuously effective.

There may exist cases of continuous variation purely phenotypic, as that of Johannsen's beans seems on his showing to be. In other cases phenotypic variations may so largely exceed genotypic variations that it is difficult to discover and isolate the latter, as has been Pearl's experience. But our experiments with rats show beyond reasonable doubt that genotypic variation, as well as phenotypic, may assume a continuous form, and if it does no one can question its further modifiability by selection. In denying effectiveness to selection in the case of continuous variation, it has been assumed, tacitly by DeVries and expressly by Johannsen, that continuous variation is wholly phenotypic. This assumption being disproved, the pure-line theory which rests upon it lacks adequate support.

It seems strange looking backward that the idea should have become so widely accepted that continuous or fluctuating variations are wholly phenotypic. For a continu-

ous variation signifies only the combined result of several independent agencies. In purely phenotypic variation (such as possibly Johannsen has observed) these agencies are obviously environmental and so do not affect the inheritance. But in a case of multiple genetic agencies (the existence of which everyone recognizes) a continuous series of variations may result which would be amenable to selection. Pearl and all other pure-line advocates admit the existence of such cases. But the same thing would result if, aside from purely phenotypic variations in a character, its single factorial basis should undergo quantitative variation. It is precisely this last named category of cases which alone can explain our rat results. And it is precisely this category of cases which the pure-line advocates, unable to disprove, boldly deny. Driven from all other defences they cling to this as their last line and solemnly repeat challenges issued years before in moments of greater confidence. Thus Pearl closes his paper with a renewal of the opinion expressed by him in 1912.

It has never yet been demonstrated, so far as I know, that the absolute somatic value of a particular hereditary factor or determinant (*i. e.*, its power to cause a quantitatively definite degree of somatic development of a character) can be changed by selection on a somatic basis, however long continued.

Our observations on rats are submitted as a sufficient answer to this challenge.

I do not suppose that Pearl means to be taken seriously when he says (p. 608):

The extreme selectionist appears to believe that in some mysterious way the act of continued selection, which means concretely only the transference of each selected individual from one cage or pen to another to breed, will in and of itself change the germ-plasm.

I have never heard a selectionist, however extreme, express such a view; certainly I, whose views are attacked in the next sentence, have never entertained such an idea. But Dr. Pearl knows, as well as I do, that while the germplasm of the individual remains unmodified upon its trans-

fer from one cage to another, the character of the *germ-plasm of its descendants,* and so of the race, depends very largely upon what mates are transferred to the same cage with it. This is where the selection comes in and there is nothing "mysterious" about it either.

The idea that selection can bring about no change in the germ-plasm of the race "except by sorting over what is already there," to which Pearl gives expression, rests on the assumption that the germ-plasm never changes. What ground have we for such an assumption? No more than for the idea of the unchangableness of species, which formerly prevailed. Even Johannsen admits that large germinal changes ("mutations") *sometimes* occur. He himself records having observed them. Why should we be so skeptical about the occurrence of minor germinal changes? It is easy to overlook them when purely somatic changes are associated with them and outnumber them as they possibly do in Johannsen's beans and Pearl's fowls but a single clearly established case should suffice to establish their existence and their importance in evolution.

THE INHERITANCE OF BLACK-EYED WHITE SPOTTING IN MICE

C. C. LITTLE

Black-eyed white varieties of rodents have long been recognized and used as material for genetic investigation. Cuénot, Morgan and Durham with mice and Castle with guinea-pigs have utilized this particular color variety in breeding experiments. For the most part they are agreed that black-eyed white varieties represent an extreme condition of the ordinary "spotted" or "piebald" series.

Cuénot (1904) in treating the inheritance of spotting concludes that there exists a continuous series of partially pigmented forms extending on the one hand from mice with white on the tail, or with a small white ventral patch, or with small white forehead spot, through a series of decreasingly pigmented forms until the black-eyed white form is reached at the other end of the series. As to a factorial explanation for the phenomena observed in the inheritance of spotting, Cuénot feels that there are numerous stages of the spotted condition (P) which he designates by p^1, p^2, p^3, p^4 as progressively whiter forms are considered. He believes, however, that the details of spotting are not represented in the germ cell. He further mentions the failure to obtain any particular stage of spotting in a true breeding condition. Selection of nearly solid-colored forms has enabled him to obtain animals with greatly increased white areas.

Durham (1908) has obtained some evidence for two different types of spotting, one recessive to solid-coated forms and one dominant to them. She has reported several crosses which I have considered in more or less detail in another paper (Little, 1914). None of the crosses presented by her can be considered as critical tests of the presence of two distinct spotting factors. Morgan (1909),

who has worked with the same types as Durham, feels uncertain as to the real significance of black-eyed whites and as to the occurrence of a distinct factor for dominant spotting. This uncertainty I also felt and have tried to show further reasons for not considering Miss Durham's work as establishing the existence of a dominant spotting factor.

Castle (1905) has found that in guinea-pigs black-eyed whites behave in inheritance in much the same way that the same type of mouse behaves, namely that black-eyed whites do not breed true but give, when crossed *inter se*, a whole range of spotted forms in addition to some like themselves.

One can by selection progress in either direction through a series of spotted forms, decreasing or increasing the number and extent of pigment patches. Great difficulty, however, was encountered in trying to fix the color pattern at any particular stage in the series. Up to the present time this has not been proved possible.

EXPERIMENTAL

In the early winter of 1913 Dr. Castle obtained from a fancier in England two pairs of black-eyed white mice. These he kindly handed over to me for investigation. From the outset the progeny of these mice proved to be extremely healthy and vigorous.

1. *Black-eyed White Crossed Inter Se*

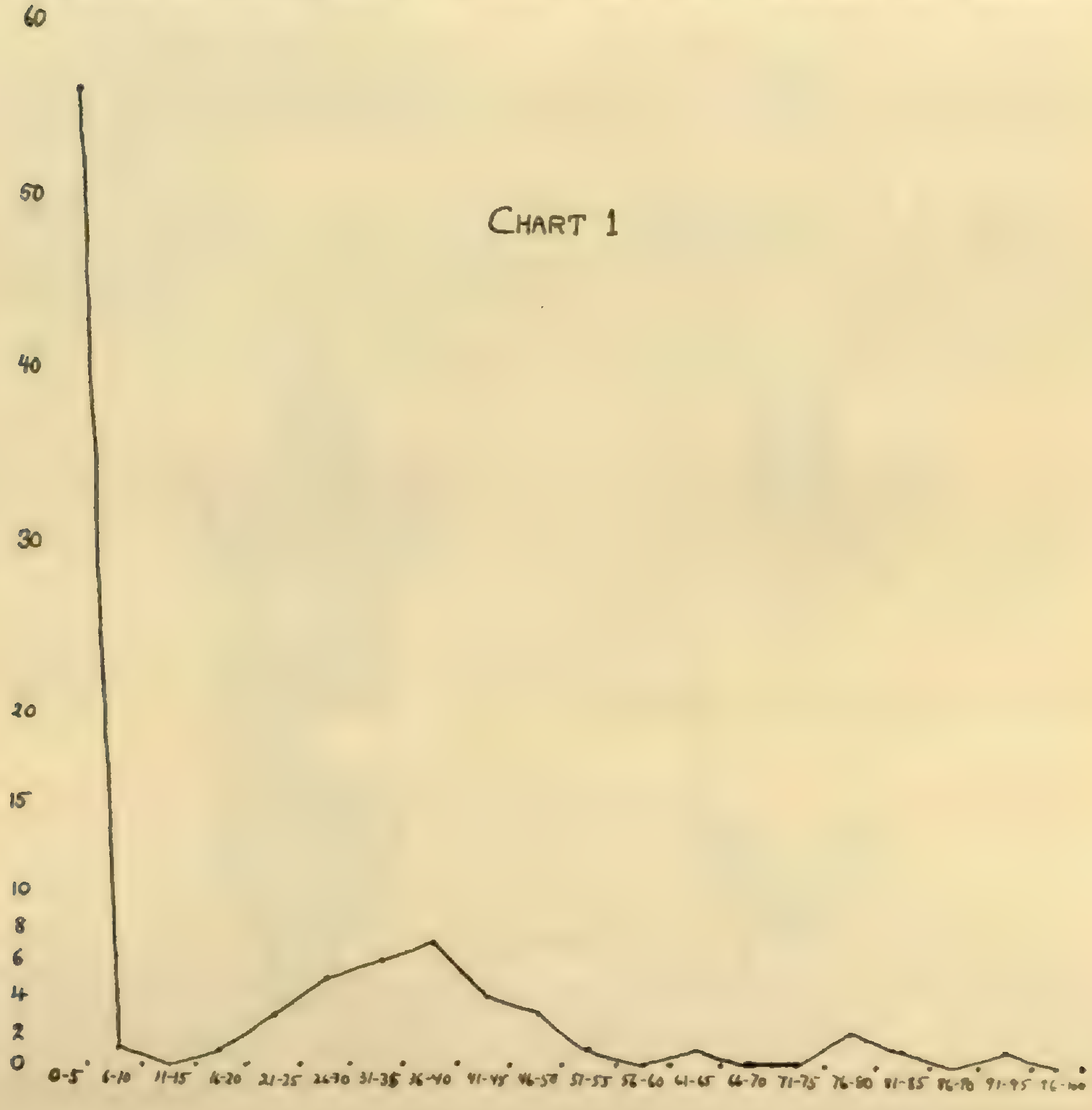
This cross gave two distinct classes of young, black-eyed white and "piebald." The distinction between the two classes can best be shown by the tabulation of their progeny on the basis of the amount of dorsal pigmentation they possess. I have for some time estimated the per cent. of the dorsal surface pigmented in the case of all spotted animals recorded. This gives a basis for classification which, though it may at first glance seem to inexact, nevertheless has been shown by comparing the

estimates of two or more investigators on any one animal to be surprisingly exact and fully as satisfactory as any other system of grading.

TABLE I.

Type of Cross	Per Cent of Dorsal Pigmentation																			
	0–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70	71–75	76–80	81–85	86–90	91–95	96–100
Black-eyed white *inter se*	56	1	0	1	3	5	6	7	4	3	1	0	1	0	0	2	1	0	1	0
Black-eyed white × piebald	105	0	0	1	1	12	8	11	5	6	4	4	1	3	4	9	4	9	3	0
Piebald × piebald	0	0	0	0	0	4	6	7	13	6	2	9	4	6	2	5	8	15	2	0
Total	161	1	0	2	4	21	20	25	22	15	7	13	6	9	6	16	13	24	6	0

From Chart I it will be seen that 44 of the 75 young obtained fall in the class between 0 and 5 per cent. of dorsal pigmentation. These are the black-eyed whites.



The remaining 31 young are more or less scattered along the range of "piebald" forms. The gap between the two classes is a considerable one and is certainly significant.

FIG. 1

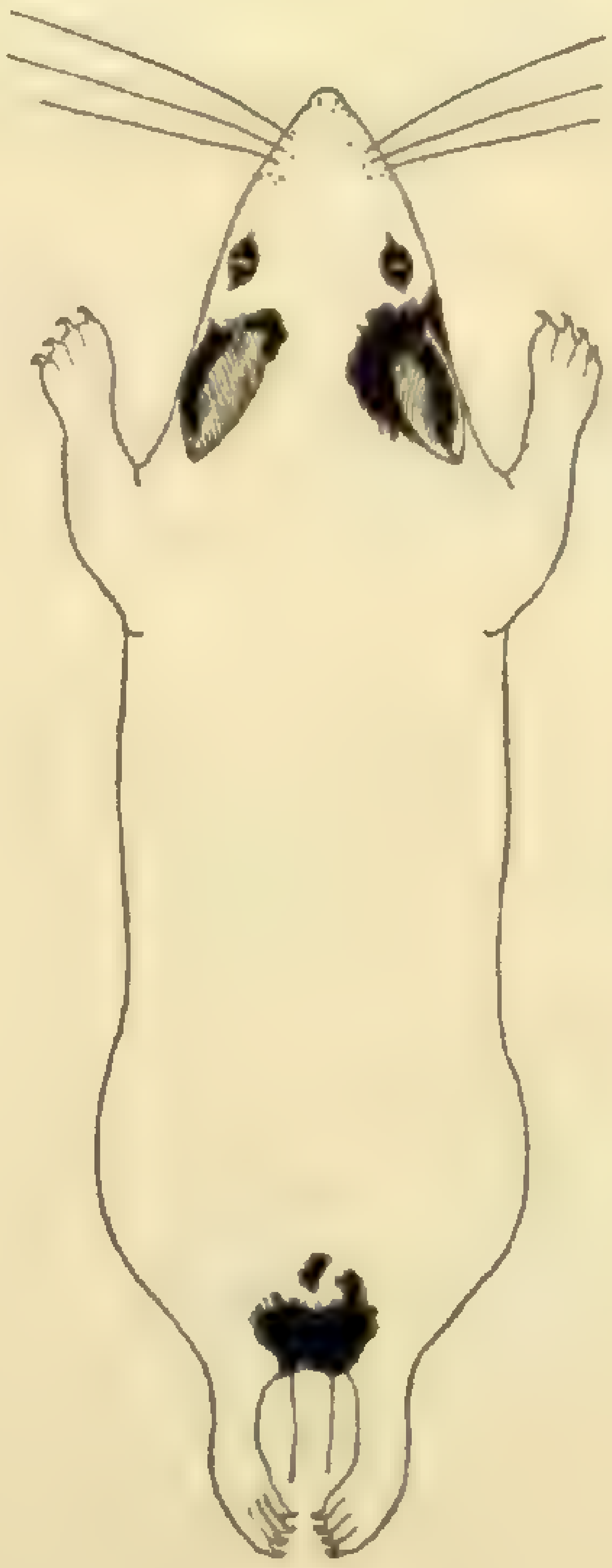


FIG. 2

Figs. 1–4 are diagrammatic and are intended to show the two groups of spotted animals. Figs. 1 and 2 show

FIG. 3

FIG. 4

the common range of variation within the black-eyed white type and Figs. 3 and 4 the same for the "piebald" type.

2. *Black-eyed White* × *Piebald*

This mating brought out two interesting facts. *First,* all black-eyed whites behaved in essentially the same way, approximately an equal number of black-eyed white and piebald young being produced. *Second,* the same distinctness between the two types held good, as will be seen from the chart given below (solid line).

3. *Piebald* × *Piebald*

Piebald animals from black-eyed white parents and from the cross of piebald × black-eyed white were mated *inter se. They produced only piebald young, 93 in number.*

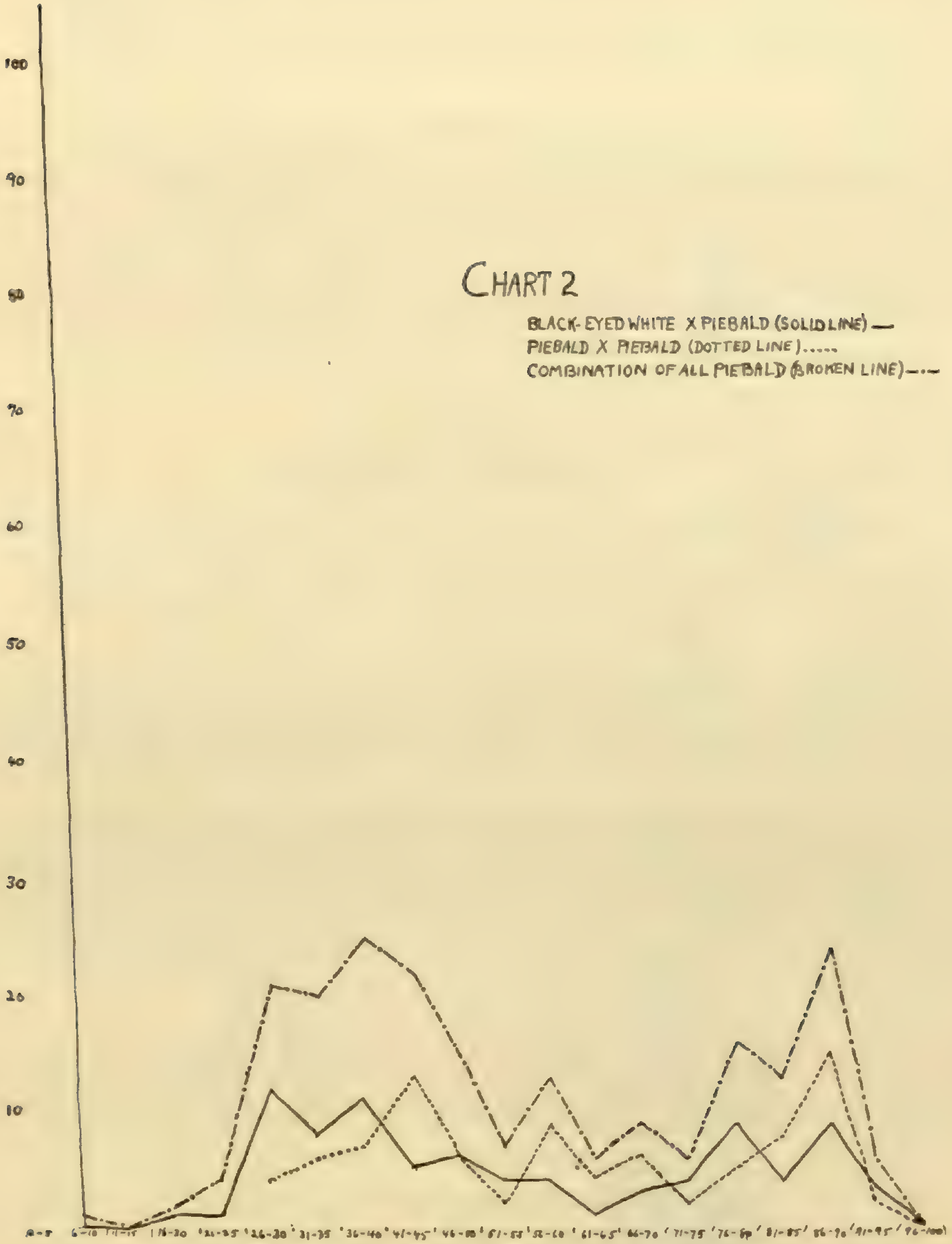
The distribution of these young according to the degree of dorsal pigmentation they possessed is shown by Chart 2 (dotted line).

It will be noticed that there is no approach to the black-eyed white condition (0–5 per cent.). There are also indications of two main modal points, one at 41–50 per cent. and one at 80–90 per cent. A complete curve formed from the sum of all piebald animals included in Table I, is given in Chart 2 (broken line).

This further emphasizes the bi-modal nature of the curve in the case of piebald mice and makes it seem likely that there are two genetically distinct grades of this variety. It is hoped that opportunity will arise in the future to investigate this point more accurately.

4. *Discussion*

From the three types of matings given above the following facts may be deduced: (1) The inheritance of the characters in question is alternative, not blending in nature; (2) black-eyed white is epistatic to ordinary piebald spotting.



The behavior of black-eyed whites in crosses 1 and 2, Table I indicates that they are always heterozygous dominants and that they can not, therefore, be obtained in a condition to "breed true."

With this in mind it is interesting to calculate the expected ratio when black-eyed whites are crossed *inter se*. If black-eyed white is due primarily to a dominant factor

which obeys the ordinary laws of mendelian inheritance, we should expect that black-eyed whites would be obtained of two genetic types, homozygous and heterozygous. If now black-eyed whites were mated together at random, the matings should be either (1) DD × DD, (2) DD × DR or (3) DR × DR. In the case of (1) and (2) only black-eyed white young should be produced, while type (3) should give approximately 3 black-eyed whites to one piebald. Random matings would therefore produce a ratio of black-eyed whites to piebalds considerably in excess of 3:1.

If, on the other hand, the DD form of black-eyed white mice behaves in a fashion similar to the homozygous yellow mice, failing to develop, we should expect a ratio of 2 black-eyed whites to one piebald young, no matter what the origin of the black-eyed white parents might be, whenever two black-eyed whites are bred together.

The results are as follows:

Black-Eyed White × Black-Eyed White

	Black-eyed White	Piebald
Observed	57	39
Expected 2:1 ratio	64	32
Expected 3:1 ratio	72	24

When one realizes that the ratio in one case should be considerably *higher* than 3:1, it seems that the results indicate a 2:1 ratio and the heterozygous nature of black-eyed whites.

To further test this hypothesis individual tests of twenty-one black-eyed whites coming from black-eyed white parents were made by crossing with piebald animals. If the DD combination is possible, approximately seven of the twenty-one tested should be of that constitution. All of them, however, proved to be heterozygous. While the numbers should be supplemented by further tests, they are certainly sufficient to serve as a basis for a tentative conclusion that black-eyed white mice are always heterozygous.

The numbers from the cross of piebald × black-eyed white are more extensive and closely approximate a 1:1 ratio. The numbers obtained are 105 black-eyed whites and 102 piebald, while the 103 of each would have been exactly an equality ratio.

The behavior of the piebald animals when crossed *inter se* is exactly what would be expected if piebald was hypostatic to black-eyed white and distinct from it in inheritance.

The next question to be considered is the relation of black-eyed white to "self" or solid coat, in inheritance.

Relation of Black-eyed White to Self

A preliminary investigation of this question has been made. The "self" race used was really technically not a "self" but genetically it carried neither the black-eyed white nor piebald spotting factors. Somatically the self race used was a "blaze" race of the type which I have previously put on record. Further crosses which I have made between black-eyed whites and true selfs have shown, even in early stages, clear evidence that the behavior of the blaze and true self races is directly comparable.

1. *"Self" × Black-eyed White*

The F_1 generation produced by crossing self (blaze F6B) animals with black-eyed whites consists of two very distinct forms. These have been produced in a ratio of 50 Type "A" to 47 Type "B." The first of these, Type "A," is shown in Fig. 5. While the percentage of dorsal pigmentation of this type is subject to some variation (see table), it will be noticed that they are ordinarily between 80 and 90 per cent. colored. The spots of color

Type "A"	36–40	41–45	46–50	51–55	56–60	61–65	66–70	71–75	76–80	81–85	86–90	91–95	96–100
Black-eyed white × self (blaze)	0	1	2	0	1	1	2	0	9	15	10	8	1

appear to have slightly more irregular and less clearly defined outlines than do those of the ordinary piebald mice and many of the spots are distinctly smaller in size (compare Figs. 3, 4 and 5). Just how much of this ap-

FIG. 5

FIG. 6

pearance is due to true genetic difference between the two types of spotting is of course problematical and must remain so until a larger mass of data is available.

Concerning class "B" (Fig. 6) little need be said save that they appear in every way identical with heterozygotes ordinarily obtained in a cross between "self" and "piebald" animals. They vary from entirely solid colored animals to those having approximately 20 per cent. of the *ventral* surface white. They may be tabulated as follows:

	Per Cent of White on Ventral Surface						
	0	1–5	6–10	11–15	16–20	21–25	26–30
Type "B"	5	28	8	3	2	1	0

2. *Type "A" Animals Crossed Inter Se*

Type "A" animals obtained in F_1 are distinctly "spotted." They have a clearly discernible amount of white

and are not in the least like heterozygous "selfs" of any recorded type. When crossed together they give three somatically distinct classes of young, "self," "piebald" or like class "A," and black-eyed white. The numbers obtained are 15 "self," 31 spotted (piebald or like class "A") and 11 black-eyed whites.

3. *Type "A" × Piebald*

To test them further type "A," animals of this class were crossed with homozygous piebald mice extracted from the black-eyed white crosses. Again three general classes of young were obtained as follows: 45 "self," 54 spotted (piebald or like type "A") and 29 black-eyed whites.

4. *Type "B" × Piebald*

To compare the behavior of types "A" and "B" this cross was made. Only two classes of young resulted as follows: 82 class "B" and 78 piebald. No black-eyed whites were obtained.

Discussion

The question now arising is whether the factors for self, black-eyed white, and piebald are allelomorphic or independent in inheritance.

From the nature of the F_1 generation it is certain that the black-eyed white animals are forming two kinds of gametes in respect to their spotting factors.

If now the conditions "self" coat, "black-eyed white" and "piebald" are all related as members of a system of triple allelomorphs, we can express the cross as follows:

S = self factor.
W = black-eyed white factor.
sp = piebald factor.

Then

	S S = self	× Wsp = black-eyed white
gametes	S	W
		sp

F_1 Generation S W = Type A, Fig. 5
S sp = Type B, Fig. 6

If now animals of Type A are bred *inter se* we should expect

S | W × S W
1 SS = self
2 SW = like Type "A"
1 WW = (not formed because homozygous)

The one WW individual could not be formed since by experiment it has been shown that W can exist in only one of the two gametes forming a zygote. When W meets S, an animal like Class A is produced, when it meets sp a black-eyed white results.

The expectation therefore is that, if a system of triple allelomorphs is operative here, we should have *no black-eyed whites formed from mating together class "A" animals.*

The result of this mating quickly settles the above hypothesis for 15 "self" colored, 31 spotted (like or nearly like Type "A"), and 11 black-eyed whites have been obtained.

It is clear, therefore, that "black-eyed white" depends upon a factor which is at least partly independent of that producing "piebald" spotting. Let us suppose that this is the case and that "black-eyed whites" always carry piebald in all of their gametes and an epistatic inhibiting or restrictive factor producing increased whiteness in one half their gametes. If W equals restrictor and w its absence and sp equals the factor for piebald spotting, all black-eyed whites will be Wwspsp, in zygotic formula and will form two sorts of gametes, Wsp and wsp.

This will account for the results in mating black-eyed whites *inter se* due to the failure of the WWspsp zygote to continue its development because of the double dose of W.

If now black-eyed whites Wwspsp are crossed with selfs wwSS, two classes of F_1 zygotes will result, WwSsp and wwSsp. The former will produce a new zygotic combi-

nation really differing from the black-eyed whites in the substitution of a "self" bearing gamete for a "piebald" one in the zygotic formula. The result is an animal like Type "A," Fig. 5; Type "B," Fig. 6 shows the other F_1 type which is entirely free from the W factor and which is merely a heterozygote between "self" and "piebald."

If class "A" animals are crossed *inter se* we should on this new hypothesis expect the following results.

1	WWSS	not developed
2	WwSS	very dark spotted
2	WWSsp	not developed
4	WwSsp	like parents (type "A")
1	WWspsp	not developed
2	Wwspsp	black-eyed white
1	wwSSp	"self"
2	wwSsp	"self" or "self" with white ventral patch (type "B")
1	wwspsp	"piebald"

Four of the 16 zygotes in F_2 would have two doses of W and would not develop. Of the remaining 12, seven would have some degree of white spotting depending upon whether they were WwSS, WwSsp or wwspsp in formula; three would be "solid" colored or like type "B" of F_1 and two would be black-eyed whites.

On this hypothesis the F_2 generation would be as follows:

	Observed	Expected
Solid	15	15
Spotted	31	35
Black-eyed whites	11	10
	57	60

A further test of the nature of type "A" is possible. If they are bred to piebald animals, four classes of young should result as follows.

WwSsp	like class "A"
Wwspsp	black-eyed whites
wwSsp	solid colored
wwspsp	piebald

Lumping together the WwSsp and the wwspsp animals

we should have 2 spotted, 1 black-eyed white and 1 self. The results are as follows:

	Observed	Expected
Spotted	54	64
Self	45	32
Black-eyed white	29	32
	128	128

Whether the excess of "self" animals is significant is, of course, a question to be borne in mind but it is extremely doubtful whether it is due to anything more than a chance deviation.

Type "B" animals have, upon mating with "piebald" individuals, given very close to the expected ratio of 1 type "B" to 1 "piebald." The exact numbers are 82:78; expected ratio 80:80.

Is Black-eyed White in Mice an Allelomorph of Albinism?

The experiments of Castle and Wright have shown that a dark red-eyed variety of guinea-pig exists which is an allelomorph of dilute pigmentation and of albinism. This possibility in the case of mice is eliminated by crossing black-eyed white with albino, when on the supposition that the condition found in guinea-pigs holds true in mice all the young should be either black-eyed white, albino or dilute pigmented. Actually there were obtained from a single mating of this sort five young, all intensely pigmented, two blacks and three browns; thereby eliminating the possibility that black-eyed white, in mice, is an allelomorph in the albino series.

Conclusions

The fact that black-eyed white spotting in mice appears to be due to a factor independent of and supplementary to the factor for "piebald" spotting leads to interesting speculation as to the nature of spotting and

indicates that spotting in mice is dependent upon more than one pair of clear-cut mendelizing factors. Modifying factors which may be more or less difficult to analyze but which nevertheless are certainly present, contribute to the extent of variation in spotted races.

"Blaze" or forehead spotting is apparently independent of ordinary "piebald" spotting, as I shall hope to show in a future paper; "black-eyed white" is primarily due to an independent genetic factor and "piebald" makes a third independent type. If now in the "piebald" stock there exist at least two genetic races as are indicated by the curve of all piebald animals obtained in the "black-eyed white" crosses, the condition is still further complicated. At all events one can truthfully say that the distribution of pigment occurring as it does along a series from "self" colored to "black-eyed white" animals, offers a field for the activity of many mendelizing factors. There is no *a priori* reason why this should not be true, there are many experimental reasons steadily increasing why it appears to *be* true.

Spotting in rodents is tempting as genetic material because of the clear patterns and contrast between colored and white areas. It is, however, *as a character* extremely sensitive to minute quantitative and qualitative changes and its apparent genetic simplicity is a snare and a delusion.

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THE F_1 BLEND ACCOMPANIED BY GENIC PURITY

A Description of Mechanical Charts for Illustrating Mendelian Heredity in Each of Three Well-known Cases of Blending Inheritance in the First Hybrid Generation

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The mechanical charts herewith figured are the first of a series prepared for the purpose of presenting graphically and schematically the established facts of heredity. These particular mechanisms, illustrating blending inheritance, consist essentially of wooden slabs on which the gametic formulæ of the several generations are charted —those for P_1 and F_2 are written on flat surfaces, while that for F_1 is inscribed on cylinders which turn freely. A capital letter represents a gene; the corresponding small letter the absence of that gene. The location of genes, whether they lie in the same chromosome *i. e.,* are linked, or in different chromosomes, is shown graphically by placing their symbols in the same or in different squares, or upon the same or different half-cylinder surfaces. In each of these selected cases the individuals of the P_1 generation are homozygous in respect to both of the traits or allelomorphic phases concerned. The genes contributed by the P_1 generation to the F_1 zygote are charted on the starred faces of the freely turning cylinders. The back of each spool contains the same inscription as the face of its partner cylinder. Each face of a cylinder represents a chromosome—the two faces the two chromosome types in reference to the

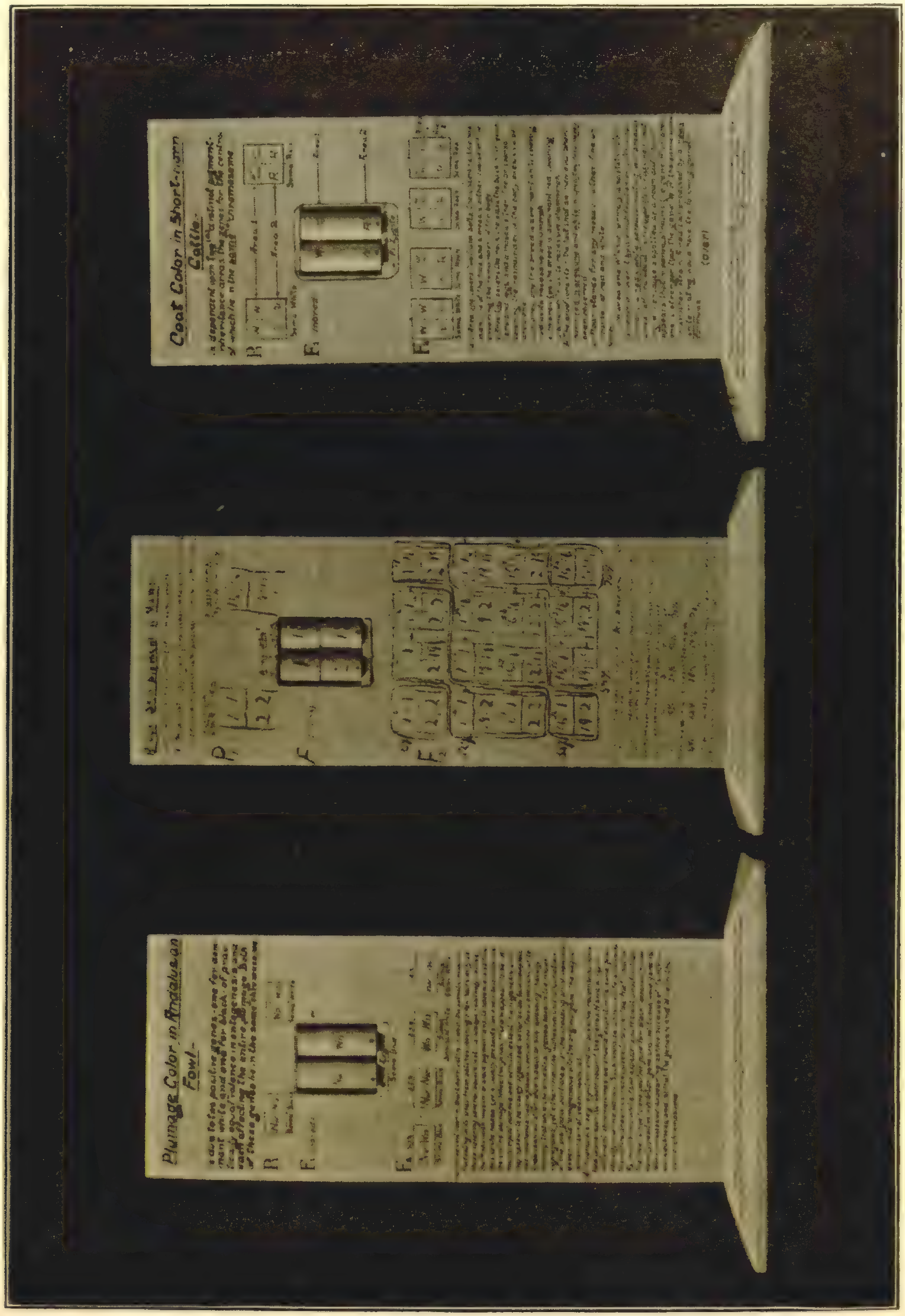

FIG. 1. Three Mechanical Charts demonstrating Mendelian Processes in Cases of Blending Inheritance

traits lying in that particular chromosome, which each F_1 individual as a parent is capable of passing on. Therefore, by turning the spools so that all possible combinations are made, one can read off directly all of the different hereditary potentialities to be had by inbreeding the F_1 generation. Consequently the F_2 line (which is charted on a flat surface) is simply a record of such combinations.

For the purpose of this study a case of blended inheritance is one in which the development in F_1 of a given somatic trait—regardless of whether it develops from one or more genes—is about midway between its development in the two parents, each of which is of pure stock in reference to the trait concerned. Until about the year 1910 students of heredity were unable to coordinate the general rule of dominance and segregation on one hand, with the frequent exception of blending and segregation on the other. Now the existence of at least three different routes by each of which nature arrives at the somatic blend in F_1 are recognized, and each finds ready interpretation in consonance with the theory of the pure gene. The first of these is the dilution or true blend route, by which nature appears to travel in the classical cases of the Blue Andalusian[1] fowl resulting from the crossing of splashed-white and black parents, and of the pink four o'clock (*Mirabilis jalapa*) resulting from the crossing of red and white parents.

The ordinary mode of inheritance is strongly duplex—that is, the zygote normally possesses two genes for each trait, either one of which genes is usually sufficient—with possibly a liberal surplus of valence—to give full somatic expression to its correlated trait. In such cases complete dominance in F_1 and clear-cut segregation in F_2 are the rule. Occasionally, however, in cases wherein a duplex parent possesses a strong somatic development of a trait,

[1] "Mendel's Principles of Heredity" (3d Impression, 1912), p. 51, by W. Bateson.

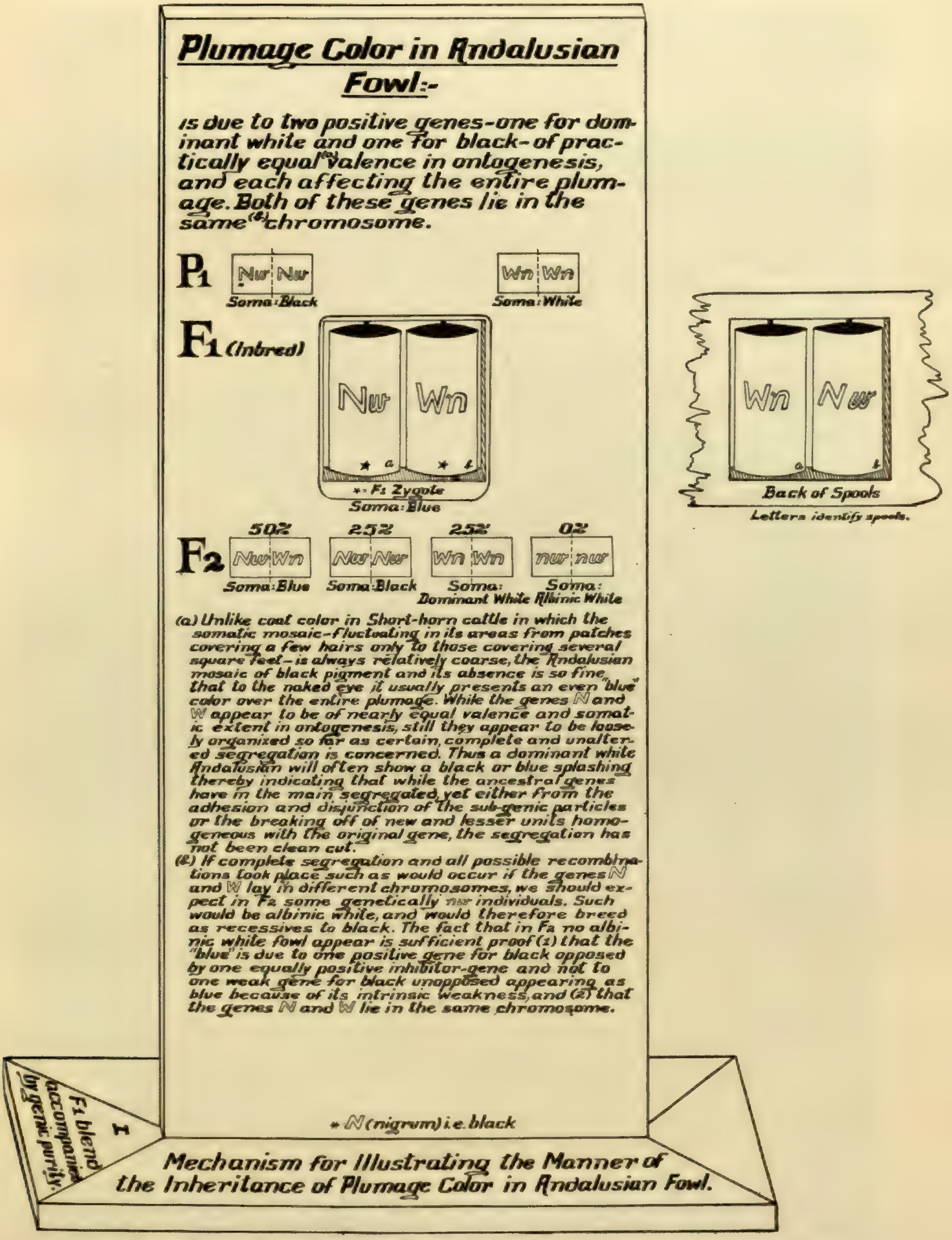

FIG. 2. Chart showing the F_1 Blend Associated with Genic Dilution—the True Blend

a single gene—from the paternal or the maternal line only—for such trait, in the zygote, is not sufficient to give a somatic development of the trait equal to that possessed by the duplex parent. In such cases, therefore, the unit trait in question is blended in the F_1 soma—a case of imperfection of dominance.[2] Nevertheless, in such cases segregation is just as clean-cut in the germ-plasm as it is in the cases accompanied by strong somatic dominance.

In Andalusian fowl "W"—dominant splashed-white—and "N"—(nigrum) black—are two opposing and allelomorphic genes of nearly equal valence in ontogenesis. Their combination and interaction determine plumage-color in the offspring. The black Andalusian is duplex for black plumage-pigment, while the splashed-white is duplex for dominant splashed-white. The F_1 offspring are "blue"—a shade really intermediate between the white and the black. Moreover, the genes "W" and "N" evidently lie in the same[3] chromosome. The evidence for this consists in the fact that in the F_2 generation, resulting from inbreeding two blue Andalusians, neither albinic white nor jungle[4]—pure or modified—patterned fowl result, which would be the case if "N" and "W" lay in different chromosomes, permitting, in some F_2 zygotic combinations, the elimination of both "N" and "W." For further explanation of this particular type of blended inheritance see the accompanying figure descriptive of the mechanical chart "Plumage-Color in Andalusian Fowl."

The second type—that of multiple factors—is typified by the inheritance of black skin-pigment in man. It is a matter of common knowledge that a mulatto of the first generation is about intermediate in density of black skin-pigment between his white and his black parents. In 1913

[2] "Imperfection of Dominance," *American Breeders Magazine*, No. 1, Vol. 1, p. 39, 1910, by C. B. Davenport.

[3] "Heredity and Sex," p. 93 et seq. (Columbia University Press, 1913), by Thomas H. Morgan.

[4] "New Views about Reversion," *Proceedings of the American Philosophical Society*, Vol. XLIX, No. 196, 1910, by C. B. Davenport.

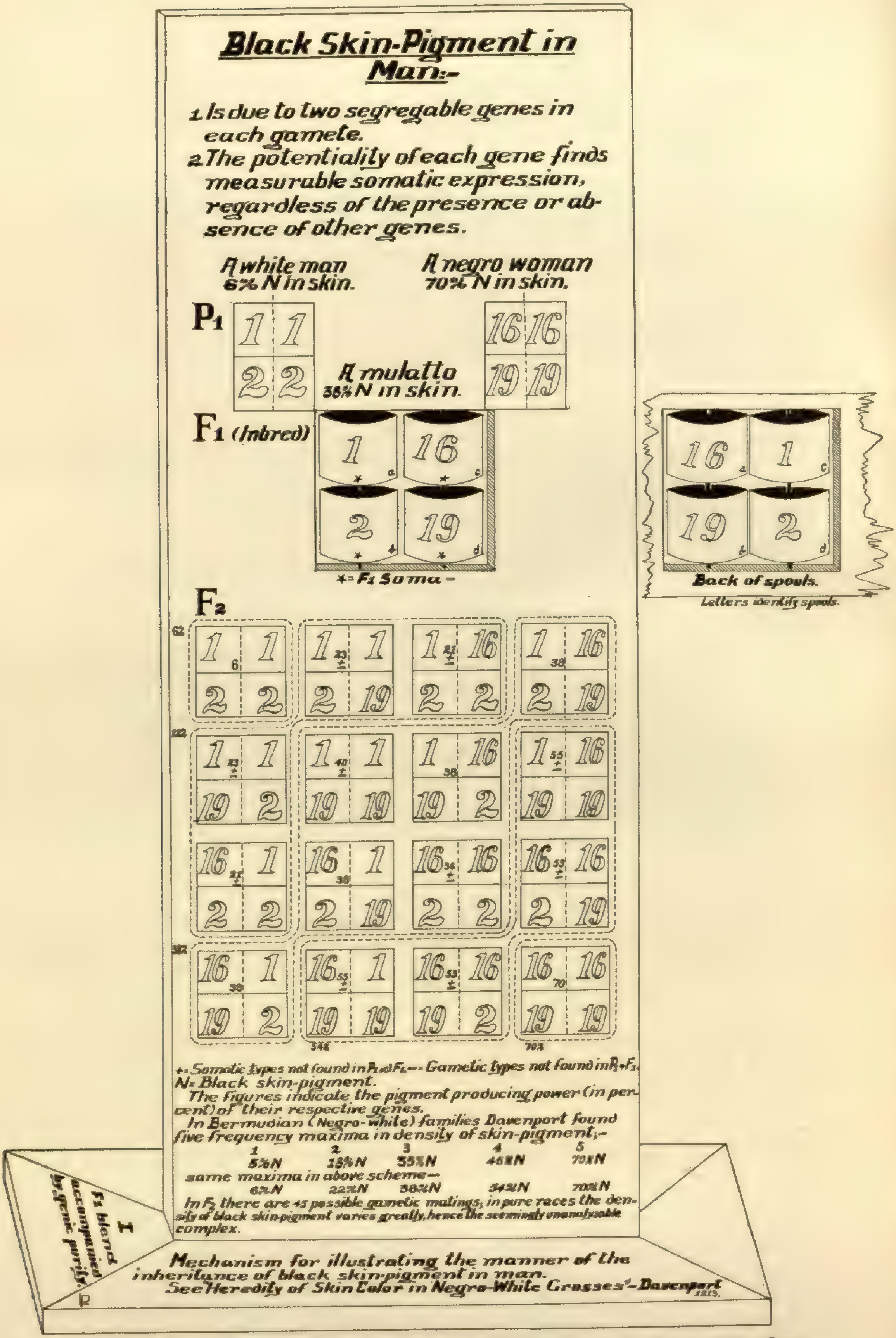

FIG. 3. Chart showing the F_1 Blend Associated with Multiple Factors for One Somatic Trait

Dr. C. B. Davenport[5] found, by analyzing data on the family distribution of black skin-pigment measured quantitatively (by the color-top) among the mixed white-and-black families of the Island of Jamaica, the Island of Bermuda, and in our own Southern States, (1) that black skin-pigment in man is the somatic working out of two segregable genes in each gamete, and (2) that the potentiality of each gene finds definite measurable somatic expression, regardless of the presence or absence in the zygote of other genes. Now these two genes appear to be of different valence; they appear also to lie in different chromosomes. The scheme outlined by the mechanical chart "Black Skin-Pigment in Man" is quite consonant with the facts of inheritance which Dr. Davenport found in nature. The facts seem to be that in white persons one of these genes will develop from practically none to about 1 per cent. of blackness in skin-color, and the second from very little to about 2 per cent., thus resulting in a blackness of skin-color of 6 per cent. or less in the somas of members of the light races. He found that some races of negroes show about 70 per cent. black in skin-color. In such races one gene for black skin-color seems to be potential to developing approximately 16 per cent. of black skin-color, the other about 19 per cent. The evidence that there are two such genes, and that they are segregable, *i. e.*, that they lie in different chromosomes, and that their values among the strains studied are about as described above, lies in the fact that, in the hybrid families in Bermuda, Davenport found 5 frequency maxima in intensity of black skin-pigmentation, and that his analysis of the family distribution of this trait, quantitatively measured in many mongrel families of known pedigree, demanded the existence in nature of the scheme above outlined.

Darwin, whose method of study was essentially observational, knew that the F_1 generation was quite generally

5 "Heredity of Skin-Color in Negro-White Crosses," published by the Carnegie Institution of Washington, 1913, by Charles B. Davenport.

remarkably uniform, but among and beyond the F_2 general observation found no rule of inheritance. It remained for the application of the analytical or Mendelian study to discover order in the apparent somatic tangle of F_2. The skin-color story just related is a striking case in point.

The third class of blended inheritance—the particulate or mosaic—is typified by the behavior in heredity of coat-color in short-horn[6] cattle in which, in the F_1 soma, the

FIG. 4. Composition of Skin-pigmentation in Representatives of Three Races.

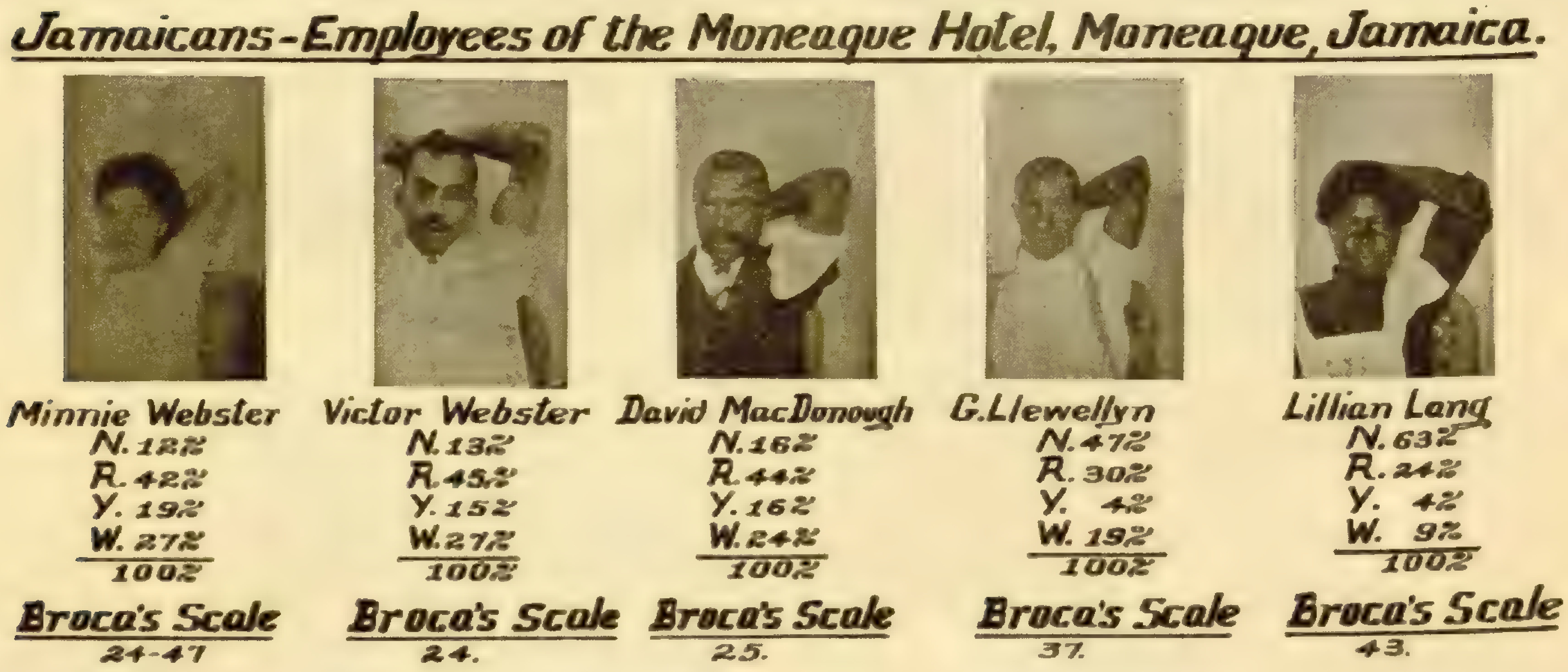

FIG. 5. Variation in Skin-pigmentation Among Jamaicans

[6] "Inheritance of Coal-Color in Short-horn Cattle," AMERICAN NATURALIST, December, 1911, January, 1912, by H. H. Laughlin.

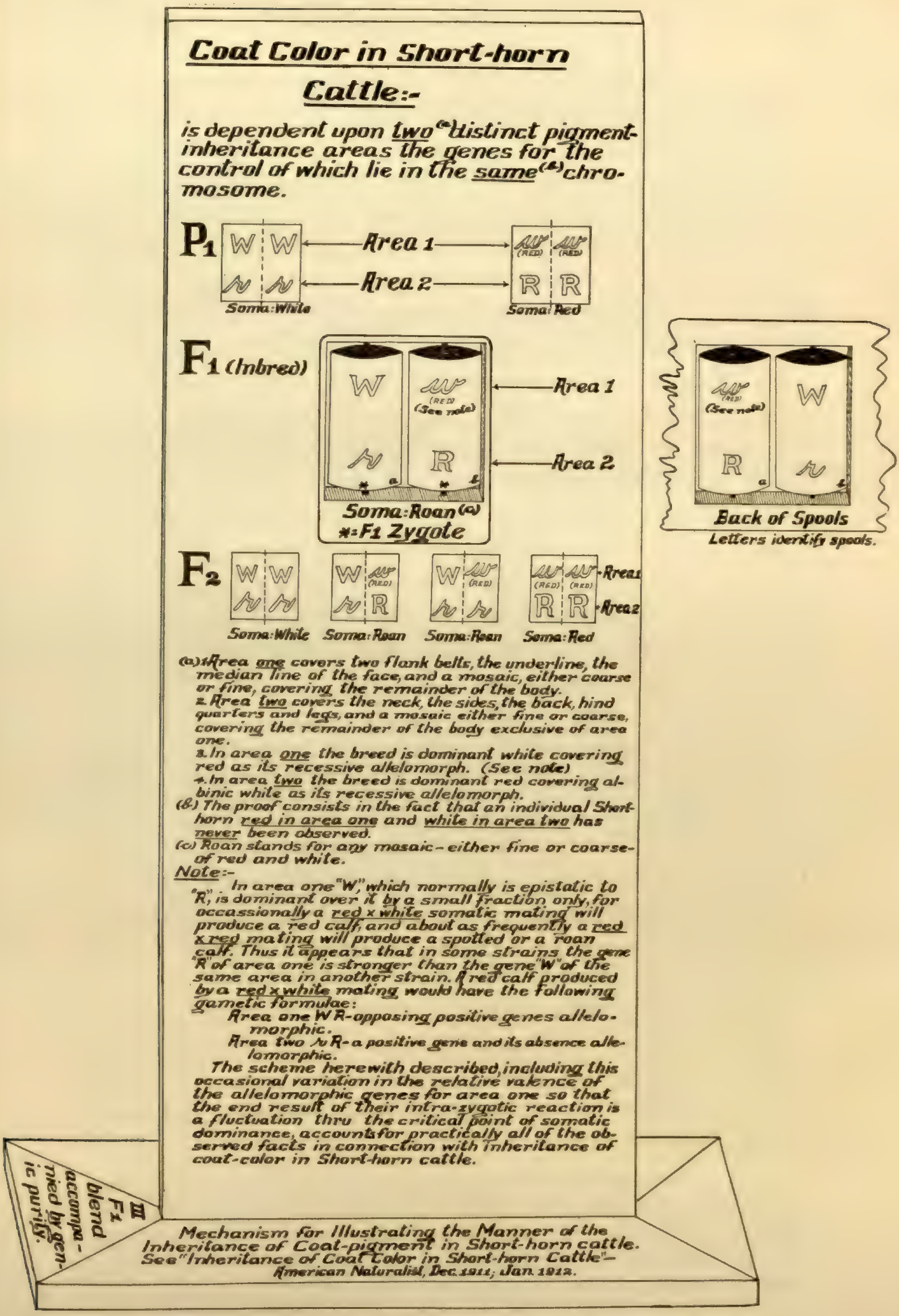

FIG. 6. Chart showing the F_1 Blend Associated with Particulate Inheritance—a Patent Mosaic

character concerned is, in its grosser aspect, clearly midway between the corresponding traits of its two parents, although a closer inspection reveals a mosaic the elements of which are the parental traits quite unchanged. The difference between the Andalusian fowl and the short-horn cattle cases seems to be as follows: In the Andalusian each gene influences the entire plumage-color, and appears to be struggling unsuccessfully, as it were, for the supremacy in somatic expression, thus resulting in a very fine and quite generally distributed blend or mosaic; while in short-horn cattle the controlling genes are double the number, each pair being confined to specific coat areas in somatic expression, and the resulting mosaic, although quite variable in coarseness, is always relatively coarse and is also quite definitely patterned.

Thus, normally (for the exception see the note in Fig. 6) in Area 1 the gene "W" is clearly dominant over the gene "R." In Area 2 the gene "R" is dominant over its absence. There seems to be in Area 2 no competing or allelomorphic gene whatever—it is simply "R" or its absence, *i. e.*, albinic white; whereas in Area 1 the "W," which is epistatic to "R," will leave "R" by its absence. The evidence for all this consists in the fact that a *white short-horn* (which is evidently dominant white, always duplex, in Area 1, and always recessive white in Area 2) will, when crossed with a black Angus, which is *dominant black for its entire coat,* give in the offspring a calf *dominant white, simplex, in Area 1,* and *black, simplex, in Area 2*—the familiar "blue roan" in cattle. That in short-horn cattle the genes "W" and "R" lie in the same chromosome is sufficiently proved by the fact that the color pattern is *never* reversed, that is to say, in *bi-colored individuals of whatever coarseness of mosaic, Area 1 is*

(Note:—When this paper on coat-color was written it was pointed out that coats red in Area 1 and white in Area 2 were *never* observed. Now the modified interpretation, involving linkage and a variation in genic valence, as explained in the text and Fig. 6 of the present article, accounts for practically all of the observed facts.)

always dominant white, and Area 2 is always red, and we never find an individual red in Area 1 and white in Area 2, although solid whites and solid reds, and bi-colored individuals of the first specified type are common. The reversed pattern, *i. e.,* red in Area 1 and white in Area 2, would occur if the genes "W" for Area 1 and "R" for Area 2 were completely segregable, *i. e.,* if they lay in different chromosomes. For a further explanation of this mode of blending inheritance see the accompanying chart, "Coat-color in Short-horn Cattle."

THE POPULATION OF THE "BLANKET-ALGÆ" OF FRESHWATER POOLS[1]

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This is a study of the community of life that is bound up with the floating masses of filamentous algæ, popularly known as "blanket-algæ." An acquaintance with this population is worth cultivating for the sake of the variety, beauty and interesting peculiarities of the plants and animals found in this unique habitat. It may be of utilitarian value as well, for there exists a relation between plankton production, algal growth and fish culture. Furthermore, it may be a help to students and to teachers of biology when they are in search of certain laboratory materials, which in these algæ masses flourish.

Method of Collecting.—A fine silk hand net of No. 12 bolting cloth was used to lift the algæ from the surface of the water. The largest collection covered about 2,800 sq. cm.; the smallest about 10 sq. cm., but most of them were from 200 sq. cm. to 800 sq. cm. in area. Doubtless, many active and comparatively large foraging animals, such as small fishes or adult insects, escaped while the net was surrounding and enveloping the mass. Probably comparatively few of the smaller forms were lost through the fine silk mesh of the net. The volume of the mass was then computed in cubic centimeters. As the mass sometimes lay in thin layers and sometimes in thicker masses, the proportion of volume to surface was seldom the same. About 200 cu. cm. was the average. The components of the "blanket" were determined and all forms, plant and animal, were listed and their size and relative abundance noted. The collections were made during the fall and early winter of 1912 and the spring and early summer of 1913.

Location and Character of the Pools.—The pools are all located in the vicinity of Cornell University campus at

[1] This study was carried on in the limnological laboratory of the department of entomology of Cornell University under the direction of Professor James G. Needham.

Ithaca, N. Y. (see map). They varied from shallow, transient collections of ditch-water to large, permanent, usually stagnant pools. Those lettered *B*, *C*, *D*, *G*, *J*, *M*, and *N* belong to the first category. Pools x, x^1, x^2, x^3, x^4,

Pools in the Vicinity of Cornell University Campus.

and y, y^1, *E*, *K*, *I*, and *L* are permanent pools and measure from four to thirty or more inches in depth. Pool *H* is a quiet part of a large stream. Pools *F* and *A* are artificially enclosed and are filled from pipes. The pools of the lowland of Cayuga Valley (about 400 ft. above sea-level) are *A*, *B*, *C*, *D*, and *E*. The others are among the hills (about 800 feet above sea-level).

The Filamentous Algæ of the Floating Mass.—Although there was such variety in seasonal conditions and in the

location and character of the pools, nevertheless some forms appeared constantly. Among the filamentous algæ, *Spirogyra* was almost uniformly present, appearing twenty-eight times out of thirty. The species were not identified until March, but in the twenty collections taken in the spring and early summer, the most frequent species was *Spirogyra varians*. *Spirogyra insignis* was found five times. Other species seen less frequently were:

S. tenuissima	*S. communis*
S. sticticum	*S. fluviatilis*
S. grevilliana	*S. bellis*
S. weberi	*S. nitida*
S. quinina	*S. inflata*
S. crassa	*S. decimina*
S. majuscula	*S. rivularis*

Usually the masses contained several species of *Spirogyra*, often with a large proportion of one species, and the *Spirogyra* was almost invariably associated with other filamentous algæ. Among the most frequent of these were *Mougeotia* and *Zygnema*. *Vaucheria* was found frequently in the autumn and early winter. *Oscillatoria* was quite constant after its first occurrence in early March, but it was usually in very small quantities. *Ulothrix Draparnaldia* and *Microspora* were seen occasionally, but not in abundance, while *Anabœna oscillaroides* was found only once. In general, the large permanent pools produced the greatest variety of genera and species of these algæ, but otherwise there was no apparent relation between the genera of algæ produced and the character and location of the pools; with the possible exception of *Draparnaldia plumosa*, which was found four times out of five in shallow ditches.

Diatoms, Desmids and Other Algæ.—Diatoms were invariably present. Of these, there were four that were constant and always in greater quantity than other kinds. These four were *Navicula*, in great variety, *Synedra*, *Cocconema* and *Gomphonema*. Other diatoms were seen ir-

regularly as to quantity and time of occurrence and included the following:

Tabellaria	*Cocconeis*
Fragillaria	*Campylodiscus*
Meridion	*Amphora*
Asterionella	*Pleurosigma*
Diatoma	*Nitzschia*
Encyonema	*Odontidium*
Cymbella	*Cyclotella*

Most of these were free but often *Gomphonema, Cocconema* and *Cymbella* were in colonies attached by branched or simple stalks to larger forms. *Encyonema* is found end to end in colonies enclosed in long filament-like gelatinous envelopes. *Navicula* as well as stalked diatoms sometimes covered the bodies of larvæ and smaller crustaceans and also the cases in which the chironomid larvæ spent part of their time. Variation in occurrence of diatoms is apparently due to seasonal changes, which will be considered later.

Other algæ were less constant, the most regular one being *Closterium,* which occurred in eight collections, showing a number of species. Of the other desmids that appeared, *Cosmarium, Penium* and *Staurastrum* were usually in small quantities. Twice, however, *Cosmarium* and *Closterium* both appeared in abundance, the first time being in a permanent but shallow pool (*I*) where *Ulothrix* predominated, and the second time in a shallow but probably permanent roadside pool (*G*) covered with *Spirogyra.* The Volvocaceæ were represented by *Volvox, Eudorina, Pandorina, Sphærella* and *Chlamydomonas.* Two Phæophyceæ, *Dinobryon* and *Synura,* and four Protococcaceæ, *Dictyosphærium, Kirchneriella, Protococcus* and *Scenedesmus,* added variety but did not appear frequently. *Peridinium, Pediastrum* and *Ophiocytium* were rare.

The pools (*L* and *Y*) that had the greatest variety in desmids and kindred forms were also rich in diatoms.

These pools are large and one or two feet deep and have thin mud overlying rock bottom. Both lie near Fall Creek.

The pools (K, x, x^1, x^3, x^4) near Cascadilla Creek presented the only specimens of *Dinobryon* that were seen. These pools are permanent and deep and have stony bottom.

It may be significant that in the low-ground pools there were few kinds of diatoms and in only one such pool (A) were there any desmids.

The Animal Population.—The floating and entangled vegetation of these masses supports a large animal population. The protozoans found were particularly varied and interesting. *Ameba, Arcella* and *Difflugia* appeared irregularly in the upland pools. *Cochliopodium* and *Mastigameba* were rare. No other Rhizopods were observed. The ciliates were not determined before March, with the exception of *Paramecium,* which was listed from the first. In the twenty collections made since March first, fifteen genera of ciliates have been observed. *Paramecium* was constant and abundant. Among the larger representatives of the group, *Coleps, Chilodon, Colpidium, Stylonychia* and *Vorticella* appeared frequently and in large numbers. *Stentor, Dipleurostyla* and *Amphileptus* were less frequent, as were the smaller members of the group, namely, *Euplotes, Halteria* and *Askanasia. Coleps* was especially noticeable in pool y^1, while *Vorticella* was plentiful in pools D and G. Pools D, G and J, which supplied the largest number of genera and of individual ciliates, are shallow ditch-pools with muddy bottom, while A and Y in which smaller numbers were found, but still many genera, are larger and deeper, but have muddy bottom or muddy water. From this it seems evident that these protozoa prefer water with inorganic material in suspension, although they are said to avoid water polluted by decaying organic matter. These tiny creatures forage busily among the algal filaments, some swimming and rotating smoothly, others, such as *Halteria* and *Stylonychia,*

moving by jerks and sudden dartings hither and yon. The minute form, *Euplotes*, has a peculiar method of locomotion that looks like walking along a filament, though it is merely forward progress by means of cilia.

The flagellates were represented by *Euglena*, *Distigma* and *Phacus*, of which the first was fairly constant. Three heliozoans, *Actinosphærium*, *Actinophrys* and *Vampyrella*, appeared infrequently. *Hydra* was found in one collection only, and no other cœlenterates were seen.

Various worms, mainly the microscopic nematodes as well as unidentified planarians and turbellarians, also three kinds of oligochætes, namely *Nais*, *Tubifex* and *Chætogaster*, were frequent but not regular inhabitants of the alga-mass.

The rotifers were regularly a part of the population, furnishing species of eighteen genera. Determination of genera was not undertaken until March The genus most constantly in evidence was *Diglena*, especially in dirty water, foraging industriously, nibbling and pulling at the algæ. A species of Metapidia with a broadly curved lorica was seen several times. It clung by its toes to debris, while the flow of water carried food-particles past the rotating cilia into the mastax. *Anurea*, *Salpina* and *Synchæta* were found exclusively in the pool nearest Cayuga Lake, pool *A*. Also in this pool, as well as elsewhere, were found *Polyarthra*, *Rotifer*, *Adineta*, *Diglena*, *Notholca labis* and a long-spined species of *Rattulus*. This permanent pool and a similar pool (X^1) of the highland were richest in rotifer life. Forms found in the latter pool and not observed elsewhere, were *Notommata* and a species of *Stephanops* with a fan-like anterior projection of the lorica. Other genera identified were *Brachionus*, *Philodina*, *Mytillina*, *Mastigocerca* and *Diaschiza*. *Chætonotus*, a representative of the *Gastrotricha*, was seen a few times.

The Gastropoda, the only mollusk group represented, did not furnish a constant element, since only eight collections contained any snails. *Lymnea* appeared once,

Physa five times and *Planorbis* four times. These snails varied in size from two to twenty-five mm. long. Except in one instance, they were in shaded pools or came out on cloudy days. The exceptional case may be considered as similar because the luxuriant growth of watercress near the algae furnished spots of shade, although most of the "blanket" was in sunlight. It seems fair to assume that snails are not regular inhabitants of the surface algæ, but merely forage there when there is little or no sunlight.

Many small crustaceans were observed. *Chydorus* and *Bosmina* were numerous, while two other Cladocera, *Daphnia* and *Simocephalus,* were less in evidence The ostracods found were in eleven collections and quite numerous. They have not been identified. *Cyclops, Canthocamptus* and *Diaptomus* were the copepods identified. *Cyclops* was remarkably constant and abundant. Many females bearing paired egg-sacs and many copepod nauplii, presumably young cyclops, were among the number. The adults were from one to three mm. long. The Isopod, *Asellus aquaticus,* was found only once and then in a mass of algæ close to a mud bank. Two amphipods, *Gammarus* and *Hyalella,* were observed several times.

The last group of foraging animals and the one to which the largest individuals of this population belong is the Insecta. In this class were found larvæ, nymphs and adults, representing five orders of insects. Three nymphs of *Callibætis,* in pool *A,* one of *Bætis,* in pool *M,* and ten of undetermined genera of *Ephemerida* in Pool *F* were the only may-fly nymphs found. The *Odonata* were more frequent. There were a few Libellulids, and a number of nymphs of *Enallagma* and *Ischnura.* The Hemiptera had only one representative, *Corisa,* the water-boatman, which was caught twice but was frequently seen swimming on the clean surface of the pool. It can hardly be considered a regular inhabitant of the alga-masses.

Four different larvæ of the order Diptera made up the greater part of the insect population. *Chironomus* was particularly conspicuous, since the larvæ were found con-

stantly, and were generally very numerous. Masses of eggs of *Chironomus cayugæ* Johannsen were found enclosed in an oval mass of gelatin anchored to some of the algæ, also myriads of newly-hatched, almost microscopic larvæ were seen, so it is reasonable to assume that, for these pale pink or yellowish chironomus larvæ (1–18 mm. long), this environment is the normal one. A few larger species, some of them blood-red, were found also. Larvæ of the "punkie" *Ceratopogon* and of the soldier-fly, *Odontomyia,* were seen occasionally. Although mosquito-larvæ are found regularly in stagnant pools, it is surprising to note that only twice were these larvæ found among the filaments of the floating algæ. These larvæ were not identified.

A few larval beetles and a few adults made infrequent appearances. Undetermined *Hydroporus* and other dytiscid larvæ were among these. Although known as a dweller among filamentous algæ, the Haliplid beetle larva, *Peltodytes,* was seen only once, its long spiny hairs tangled in the vegetation. Adults of two genera of Hydrophilid beetles were identified as *Helophorus* and *Crenophilus* and a few other diving-beetles were seen but not identified.

Although tadpoles, and once a young salamander, were found in the collections, they can hardly be reckoned as members of the society under consideration.

Dominant Forms.—In this diverse population the constant and abundant forms have been few. *Spirogyra,* especially *Spirogyra varians, Mougeotia* and *Zygnema,* were the principal constituents of the "blankets." Among the Diatoms, the dominating forms were *Cocconema, Navicula, Gomphonema* and *Synedra.* Other algæ were best represented by *Closterium, Dictyosphærium* and *Dinobryon.* Among the animals *Paramecium, Euglena* and the rotifer, *Diglena,* were quite constant. The forms that appeared most regularly were *Cyclops* and the larvæ of *Chironomus.* Some of the less constant forms showed the influence of seasonal variation.

Seasonal Variation.—In the autumn and early winter *Vaucheria* was usually present, but appeared only twice in the spring. *Pandorina* and *Peridinium* also appeared late in the year. At that time fewer protozoa were seen than in the spring, but, as has been said, variations here seem to be more closely related to the character of the water than to the temperature. *Gammarus* and the nymphs of may-flies and dragon-flies were most numerous in October, November and December.

The spring season also had its special forms. *Oscillatoria* appeared first in March and was constant thereafter. Diatom production was at its height in April and May at water-temperatures varying between 8° and 16° C. and there was a marked decline in diatom appearances toward the end of June. In contrast to diatoms, desmids seem to require higher temperatures, since most of the *Closterium* and all of the *Cosmarium* and *Staurastrum* that were seen appeared in June, in water at temperatures between 15° and 20° C. The proportion of *Dinobryon* in collections became noticeably greater during the latter part of June. The smaller crustaceans, excepting the ever-present *Cyclops*, showed marked increase in numbers as well as in diversity during May and June. The same seasonal increase was noticed for *Anguillula* and the rotifers. Most of the coleopterous and dipterous larvæ were found in May and June, except *Chironomus* which was present at all seasons.

Another point of interest in connection with seasons is the time of reproduction. *Spirogyra* was found conjugating in October, April and June; *Mougeotia* in November, December, May and June. Young, sessile plants of *Ulothrix* were seen in April and May. All through the year, copepod nauplii and female *Cyclops* bearing egg-sacs were observed. *Chironomus* eggs were found in April and early in June, while very young larvæ were abundant during April, May and June.

In view of the fact that floating algæ were found in large quantities in December, even under ice, it was sur-

prising to find some of the pools totally devoid of this kind of vegetation in spring. Pools *K, M*, x, x^1, x^2, x^3, x^4 showed this peculiarity. Their "blanket-algæ" did not reappear until May. This disappearance of surface vegetation may have been due to spring freshets, as the pools mentioned are in the flood-plain of Cascadilla Creek, although not in the stream-bed.

The Natural Balance.—Like other societies, the population of the "blanket-algæ" has its producers and its consumers, its hunters and its hunted, each readily exchanging rôles as occasion demands. The synthetic organisms include with the phytoplankton a few chlorophyl-bearing organisms of the zooplankton; that is, forms like *Euglena, Phacus* and *Distigma*, which, in sunlight, have the holophytic method of feeding (Stokes, 1895). Diatoms require nitrates, silica and some salts to make their dainty and beautifully marked shells. Since they are comparatively heavy, they sink slowly, but are brought to the surface during the spring and fall circulation of the water. In spring they multiply rapidly near the surface, since they need oxygen and sunlight.

Many of the tiny creatures, including ciliates, *Cladocera*, rotifers and nymphs and larvæ of some insects are in search of diatoms. These animals eat other tiny food particles as well as diatoms. The rhizopods, *Arcella* and *Ameba,* ingest diatoms, desmids, small protozoans and even rotifers. *Vampyrella* consumes the cell-contents of algæ. *Actinophrys* prefers the spores of algæ, but takes small protozoa. *Actinosphærium* is omnivorous (Stokes, 1895). Many of the ciliates eat diatoms and other ciliates. The food is drawn into the oral opening by means of currents of water which are directed toward the opening by the constant motion of cilia. One ciliate, *Chilodon,* has a peculiar method of feeding. It protrudes a broad flexible lip-like expansion of the anterior end and gathers up food particles with a sweep of this organ.

Turbellarian worms feed on rhizopods, ciliates and rotifers. Rotifers eat diatoms and some nibble algæ,

whereas the closely related Gastrotricha, *Chætonotus,* eats minute particles of decayed animal and vegetable matter, rarely taking diatoms.

The smaller crustaceans in general and the snails are scavengers, removing decaying algæ and bits of dead insects or other animal matter. The Cladocera, however, are said to eat diatoms and many of the smaller algæ. Ostracoda are omnivorous and often attack their own species.

Among the insect members of this society, the larvæ of the may-flies and midges are the great herbivores, although, in addition to algæ, diatoms and leaves of higher plants, consuming a great variety of vegetable substances, both living and dead. The great abundance of *Chironomus* larvæ make this genus an important factor, both as a consumer, and as food for other animals. *Chironomus* larvæ and pupæ are, in turn, eaten by dragon-fly nymphs, and other predaceous larvæ. They are of much importance as fish-food.

Dragon-fly nymphs are predatory. Some species eat back-swimmers and water-boatmen, small crustaceans and snails, coleopterous and dipterous larvæ and even young dragon-fly nymphs. The larger nymphs are eaten principally by fish, occasionally by water-birds.

This brief account of some of the feeding-habits will serve to show how much all the members of this society are dependent upon the others, and, at the same time, are in constant danger of extinction. Each form acts as a check upon too rapid multiplication of some other form. Since the most prolific animals in this population are *Cyclops* and *Chironomus,* each must have peculiarities that enable it to survive in this environment and to compete with other animals. *Cyclops* adapts itself easily to changes. Its prolific reproduction, seasonal constancy, and plasticity, give it great advantage over other small crustacea. *Chironomus,* also constant, prolific and adaptable, finds abundant food and comparative shelter among the algal filaments.

SHORTER ARTICLES AND DISCUSSION

ON PRACTICAL VITALISM

In a series of critical and polemical essays, published during the past few years in American journals by diverse authors, particularly by Jennings, the problem of vitalism has been discussed in a manner that may seem exhaustive.

There would appear to be no possibility of adducing new arguments in the matter. If in spite of this a new presentation is here attempted, it is because the author holds a standpoint entirely divergent from what has been thus far set forth in the discussion.

If it is true that the argumentation of the promoter and leader of the new scientific vitalism—Driesch—becomes at times somewhat metaphysical, it appears to me also that the criticism, as made by Jennings, tends at times to become dialectical and sophistical.

I can not otherwise characterize the tendency to efface any specific difference between the living and the non-living. By isolating at random a feature of the living and comparing it with an inorganic model one can indeed seem to show the identity of the two. But in this procedure we recognize the typical method of the ancient sophists. I can find nothing of interest, for example, in such an argumentation as the one cited below from Jennings.[1]

In a rejoinder to Lovejoy, who insists "that the same phenomena occur in a given organism in spite of profound modifications of the composition and configuration of the parts" Jennings objects that we have here

> a proposition that holds for things in general. An iron body of a certain form moves toward the earth. We may change the form in most varied ways . . . change the material, substitute lead, brass, stone . . .; it still moves toward the earth.[2]

Nothing is easier than to prove that black and white, plant and animal, man and monkey, are "fundamentally the same."

[1] A typical one for the antivitalistic criticism.

[2] AMERICAN NATURALIST, 1913, p. 395.

But does an affirmation of this sort annihilate in any way the specific difference between man and monkey, or diminish the interest of science in this specificity?

The innumerable attempts of the critics of vitalism to prove by comparison of certain isolated features that the living is nothing more than an extreme complication of the non-living fail, because the analysis in such cases is never exhaustive. One may prove that living and inorganic coincide in many points; he can not prove more.

I do not see why these points of coincidence are of more importance and interest for our conception of the matter than the points of undeniably distinctive difference, even though the latter are as yet unanalyzed.

The best way to test the validity of an idea or hypothesis is to follow it to its most extreme but logically inevitable consequences, taking these as a statement of the proposition involved.

If we follow this method in order to obtain an objective and exact formulation of the essence of vitalism (or of its antithesis, mechanism), we can say that what mechanism asserts is this: Whenever a certain configuration of matter occurs or is given, there also what we call "life" is found; or in more popular terms, the artificial production of a living organism from "non-living" matter would theoretically be possible.

Vitalism, on the other hand, is a standpoint that in last instance denies such a possibility.

It is clear that both the assertion and the negation are unprovable, and as such are matters of faith, not of emprical science.

If one attempts to give an estimate of the two from the standpoint of science, sympathy must, it appears to me, incline to the vitalistic view, since scepticism is the very palladium of exact science.[3]

It is generally overlooked that if one of the two opponents is to be reproved as aggressive, that one is the mechanist rather than the vitalist. The mechanist in asserting that he knows more than can be proved is filled with a scientific optimism of a somewhat frivolous character.

Yet it is the moderate agnostic standpoint, declaring no belief

[3] It may be objected that a negation is dogmatic to the same degree as an assertion. This may be true. But one can replace the term "negation" by some other less radical expression, such as "doubt," without altering the essence of the standpoint.

in the possibility of artificial synthesis of the living so long as that is not proved, that is subjected to ridicule as a dogmatic, obscurantist and non-scientific doctrine.[4]

The entire problem to me falls in the domain of "Natur-philosophie," that branch of our knowledge which can not directly prove the truth or logical necessity of the results of investigations made in its field; can do no more than to make them plausible; and thus give to us a genuine sensation of mental satisfaction.

There is no intention here of participating in the endless dispute above sketched; I do not know what could be added in this direction, from a vitalistic point of view, to the formulations of Driesch.[5] Our purpose is the defense of the right to a *practical vitalism,* as a method of exact empirical (although not necessarily experimental) investigation.

We do not care whether the methods demanded by such a vitalism are or can be proper also for inorganic investigation. It appears that they are not, for the mechanists oppose their "veto" in the name of exact science to all constructions of the vitalistic system, even though not fully analogous with that which will be detailed below.

Practical vitalism claims the right to be restricted in formulating hypotheses only by postulates of logic and of the general theory of knowledge, and by nothing else.

[4] The same point can be made with regard to the other aspect of vitalism —the so-called "experimental indeterminism." As to this, it must be admitted that the empirical evidence seems to favor the vitalistic standpoint. The assertion of the mechanists, that experimental indeterminism can not hold for the living, is likewise a matter of faith, and the burden of proof falls upon those who make it.

[5] I must nevertheless confess, despite my profound admiration for Driesch's work, that I find that his chief experimental foundation of vitalism, by means of his masterly analysis of certain cases of regulation, fails to produce the desired effect; chiefly because the entire argument rests upon certain experiments that are, as one may say, a lucky chance in biological investigation. It would be quite possible that no organisms having the marvelous powers of regulation and equipotentiality shown by Tubularia, the sea-urchin or *Clavellina,* should ever be discovered. Can it be admitted that a scientific proof of vitalism as the basis of biological research would therefore remain inaccessible? The argument in such a capital problem must, I think, rest on a more general basis, one resulting from an adequate analysis of essential and genuine vital phenomena. I incline therefore to consider Driesch's further analysis, as presented in his "Science and Philosophy of the Organism" as a no less valuable part of his work.

We hold as a justified demand of the theory of knowledge that every hypothesis must be fruitful; that is, it must give a number of deductions that can be verified empirically. Every hypothesis which permits us a prediction is to be considered a step in the progress of knowledge, until such time as it is replaced by a new one, more suitable or more fruitful.

Biological, and particularly embryological, investigation needs sometimes to introduce as a hypothesis for the explanation of certain empirical facts the idea of so-called "immaterial" (or in Jennings's terminology "non-perceptual") factors.

This is the chief point on which are based the recriminations of most critics of vitalism, especially of Driesch's vitalism.

The belief in such "non-perceptual" factors is in Jennings's mind synonymous with obscurantism or dogmatism. To Ritter "the vitalism . . . is the belief that organic phenomena can not be fully explained by referring them to the material elements of which organisms are composed, but that something *not really belonging to the natural order* [?] . . . is present in living things" (italics mine).

To me it is entirely obscure why the term "non-perceptual factor," employed by Jennings in a logical and consistent manner, is by him rejected as nonsense.

His formulation of the non-perceptual is very clear.

> Conditions subject to diverse physical tests will here be called either perceptual or physical.[6]
>
> A non-perceptual agent would be one which though producing at a particular time a particular physical event, was not subject to other physical tests for its presence.[7]

I have given a formulation much resembling this, of what I call the "immaterial factor" in my paper bearing that title, printed in the "Festschrift für Schwalbe."

This work was to have been published the first of August, 1914, at the very moment of the outbreak of the war. Whether it has been issued I do not know.

My definition is as follows:

> Als materiell gilt uns im allgemeinen ein Objekt unserer Erkenntniss welches eine Mehrzahl von einander unabhängiger Eigenschaften (sc. Wirkungsweisen) in sich vereinigt und sowohl in Tätigkeit als in Ruhe befindlich wenigstens gedacht werden kann.

[6] Johns Hopkins University Circular, 1914, No. 10, p. 8.

[7] *Ibid.*, p. 12.

Ein zur Erklärung bestimmter Wahrnehmungen ersonnener Factor von dem eine derartige Annahme d. h. ein Zustand der Nichtbetätigung widersinnig wäre mag folgerichtig als "nicht materiell" bezeichnet werden.

Neither Jennings's "non-perceptual" or my "immaterial" can be considered an illogical or contradictory conception. Criticism must, so far as my own doctrine is concerned, be therefore concentrated solely on the strength of the empirical foundation for the hypothesis of immaterial factors in any given case.

Besides the logical definition given above, an examination is required of the question; What exactly can be meant by, or *how can one be led to assume, an "immaterial factor" as a result of experimental investigation, or at least as a hypothesis impelled by such a result?*

To Jennings the assumption of a "non-perceptual agent" leads directly to, or is synonymous with, the so-called "experimental indeterminism," as admitted by Driesch.

He seems to neglect every other possibility of the action of an "immaterial factor." I do not see that this is inevitable.

To me the essential point of the problem lies in the question of the "bearers" for any sort of empirically detectible action (induction, force, or the like).

Suppose that it were found that the factors directing the movements of a given element of a living organism (for example, the cell of an embryo), in a given direction *m* to the point *n*, lie outside itself.

We will then assume at the point *n* a center of forces.

Suppose now that we can deduce from this assumption certain consequences that will be subsequently verified empirically.[8] Our assumption that gives us possibilities of prediction becomes then a scientific reality. We say "reality," although it may remain somewhat hypothetical. We find the same condition of affairs in the imperceptible but strongly inferred realities of physics, etc.

[8] I find that there is a point at which Jennings's conception of the "non-perceptual" seems to lead us wrong. It is well to say with Jennings that such an agent is one producing at a particular time a particular physical event *but not subject to other physical tests for its presence* (italics mine). But Jennings seems not to take into consideration that a "particular physical event" or a "single mode of action" (in my formulation) can lead to many empirically verifiable *consequences.*

Suppose now that at the point of space where we have projected the center of forces there lies some element of the embryo, such as a cell. The scientific routine will call this element the "bearer" of the forces in question.

But it is also possible that no element and no matter is to be found at this point.

The first impulse will be to search for some other element of the embryo, situated elsewhere, that can act as such a center, by irradiating certain "lines of force," which influence in some manner the movements of the first considered element. We will, however, assume a case where no such element acting at a distance can reasonably be supposed. What now?

If the fundamental assumption holds true, that the factors determining the movements of the element lie outside of itself, we find ourselves confronted by the following alternative:

Either the presumed factors have a bearer that is not cognizable, or they have no material bearer at all!

It is clear that if we deny the existence of bearers that are evidently perceptible, we can also exclude the possibility that such bearers exist, but are invisible owing to their minuteness; for the presumed center of forces lies according to our assumption outside the organism; or in a district of it where there is no formed embryonic matter at all.

Thus under the circumstances our two alternatives signify the same thing, for to say that there is a bearer of factors that is cognizable solely as the factors themselves involves a tautology; an assumption of the sort so well characterized by the French as a "hypothèse gratuite." While any one is free to make such an assumption, no scientific use can be made of it. Methodologically it is perhaps comparable to Kant's "Ding an sich," which likewise must remain without empirical content.

As a fundamental postulate of biological (and especially of embryological) research, there can therefore arise the conception of factors which, although spatial and localized in space, have no material bearers, and as such may be denominated immaterial.

Is such an idea indeed nonsense; something that proves the obscurantism of its promoter?

I am well aware that the "immaterial" factor here presented is far from coinciding with Driesch's Entelechy or with any

analogous agent that is *e definitione* not solely immaterial, but also non-spatial.

If the entire weight of antivitalistic criticism is directed and concentrated wholly against such ideas as that of Entelechy; and if the mechanist will agree with me that a spatial localization of a center of forces may be assumed without necessarily combining this with a material bearer, I shall be much gratified. But I fear that this is not the case. The "dynamical præformation of the morphe," as I have elsewhere called the immaterial but spatial factors of morphogenesis,[9] must, I fear, fall under the same anathema as the classical vitalism.

To resume the chief postulate of my own "vitalism": if morphogenetic investigation is led in a rigorous inductive way to assume a spatial factor at a definite point inside or outside the embryo, no difficulty or contradiction or nonsense arises if no "embryonic" matter, or what is the same, no material "bearer" for this factor can be found at that point. Yet of course no one can be prohibited from forming any sort of hypothesis as to such functionless bearers. It may be even a psychological necessity to form such hypothesis, for we love a "Ding an sich." But such will form no part of empirical research.

The right to work with such immaterial factors, and in the inductive way set forth above, is, for me, the essence of *practical* vitalism.

We have now to examine consequences and postulates derived from our fundamental assumption, which seem to present very great difficulty. If we admit a dynamical factor localized in space but not derived from a material bearer, it will be asked, whence comes and how arises this factor?

The question of causation is based on a postulate of knowledge that can not be eluded; it must be answered in some manner.

I will attempt to point out briefly how one can think the origin or evolution of such an immaterial morphogenetic factor, although it must be insisted that we have here a problem which does not stand in immediate connection with the purely empirical method of investigating the factors considered, so to say, *per se* in their activity.

I see no difficulty in assuming an immaterial causality; that is, the arising of an immaterial factor having a certain property

[9] *Biologisches Centrablatt*, Bd. 32; *Archiv. f. Entwicklungsmechanik*, Bd. 39; *Festschrift für Schwalbe*, 1914.

(for example, configuration) from another less complicated immaterial factor, and so on.

The chain of immaterial factors could in this manner logically be pursued backward to the beginning of the embryogenesis, or to the egg.

As to the relation of such immaterial factors to Driesch's entelechy, they can be ranged solely in the category of "means" (Mittel) of the latter for the purpose of morphogenesis.

But I repeat that this is for me a matter belonging for the present not to experimental investigation, but to the domain of "Naturphilosophie."

If it appears as if I agree in this point with the "standpoint of radically experimental analysis" of Jennings, this is not really the case. The latter author seems to reject all that does not belong to experimental investigation.

I think, on the contrary, that vigorously logical considerations, deductive and even inductive, on the given empirical data form a legitimate and integral part of our science of nature.

A. Gurwitsch

The Woman's University,
Petrograd,
April, 1915

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